OnsiteGene®

Hi-Sense™ COVID-19 Molecular Testing Kit 1.0

REF KT1010001

Instructions For Use

For use under Emergency Use Authorization (EUA) Only
For *in vitro* Diagnostic use
Rx Only

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INTENDED USE

The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 is a real-time reverse transcription polymerase chain reaction (rRT-PCR) assay intended for qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasal aspirates, nasopharyngeal wash/aspirates and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage (BAL) specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures. The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY OF TEST

Nucleic acid extraction and purification is performed using the *Quick*-DNA/RNA™ Viral MagBead Kit or the Zymo *Quick*-RNA™ Viral Kit (spin columns) from Zymo Research. The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test performed on the XDive™ Superfast Real-Time PCR System using the XDive™ Version V2.9.2 software (or higher). The COVID-19 primer and probe sets are designed to detect RNA from the Orf1ab gene and two targets (N1 and N2) from the COVID-19 N gene in upper respiratory samples and bronchoalveolar lavage (BAL) samples from patients who are suspected of COVID-19 by their healthcare provider.

BACKGROUND INFORMATION

The etiologic agent responsible for the recent pandemic of COVID-19 cases has been identified as a novel beta-coronavirus via next generation sequencing (NGS) from cultured virus or directly from samples received from several pneumonia patients. The virus was named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV). The initial outbreak occurred in Wuhan, China, but has rapidly spread around the world. The clinical spectrum of SARS-CoV-2 infection ranges from no or mild respiratory symptoms to severe acute respiratory disease and death. A typical presentation of COVID-19 disease is fever, cough and shortness of breath. Pneumonia and gastrointestinal symptoms, including diarrhea, may also occur. Severe illness with respiratory failure may require mechanical ventilation and support in an intensive care unit.

The presence of SARS-CoV-2 nucleic acid in human samples can be detected via real-time reverse transcription polymerase chain reaction (rRT-PCR) on specimens such as upper respiratory samples and BAL taken from suspected patients. RT-PCR is a laboratory technique combining reverse transcription (RT) of RNA into DNA (called complementary DNA or cDNA) and amplification of specific DNA targets. One-step rRT-PCR

mixes all the reaction components of reverse transcription and PCR together with RNA sample in a single reaction. The reaction generates fluorescent signals that can be detected in real time.

ASSAY PRINCIPLE

The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 is a Taqman®-based real-time reverse transcription polymerase chain reaction (rRT-PCR) assay for the detection of SARS-CoV-2 RNA that combines reverse transcription, DNA amplification and fluorescence detection in one step. The assay contains primers and probes designed to target two N gene sequences and one Orf1ab gene sequence of SARS-CoV-2 RNA. The human RNase P gene is included as an internal control (IC) for sample quality and fluorescence detection efficiency. In addition to the RNase P gene, the test uses both a no template control and a positive control that monitor integrity of reagents and correct performance of the testing procedure. The TaqMan® probes for the target amplicon and the Internal Control are labelled with SUN, FAM, and CY5 fluorescent dyes for Orf1ab, two N gene targets and RNase P, respectively, to generate target-specific signals.

The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 can be used to directly amplify the nucleic acid targets in the sample. The swab sample can be processed using the automated (Catalog No R2140-E/R2141-E) or manual (Catalog No R2140/R2141) Quick-DNA/RNA Viral MagBead kits, or spin columns (Catalog No D7020/D7021) from Zymo Research to extract the RNA. The sample mix can then be directly loaded onto the XDive™ Superfast Real-Time PCR system for fast detection of the virus.

KIT COMPONENTS

Packaging Specification

96 reactions/kit, Cat. No. KT1010001

Kit Components

Table 1. Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 Components

Reagents	Main Components	Qty
COVID-19 Primer and Probe Mix (300µL)	Primers and probes for the COVID-19 N gene, Orf1ab, and human RNase P.	1 tube
Superfast RT-PCR Enzyme Mix (700µL)	Reverse Transcriptase, Taq DNA Polymerase, $MgCl_2$, dNTP and buffer.	1 tube
Positive Control (PC) (3X LoD) (100µL)	150 copies per reaction *in vitro* transcribed COVID-19 N and Orf1ab sequences; RNase P gene fragments	1 tube
No Template Control (NTC) (3mL)	Nuclease-free water for the NTC	3 tubes

NOTE: Components from different lots of kit should not be mixed and used interchangeably. Integrity of kit components is guaranteed under proper storage conditions until the expiration date on the kit.

Shipping and Storage Instructions

1. The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 should be shipped with dry ice.
2. Upon receipt, all components of the kit should be stored at ≤ -20°C until the expiration date listed on the outside of the kit package.
3. Protect the Primer and Probe Mix from light during storage and handling.
4. Check the expiration date prior to use. Do not use expired reagents.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Biosafety cabinet
2. XDive™ Superfast Real-Time PCR system, Cat. No. XDIVE-16
3. XDive™ Capillary Reaction Tubes with caps, Cat. No. CM1000001
4. Sample collection kit including a synthetic fiber swab with plastic shaft and Hardy Diagnostics Viral Transport Medium (Hardy Diagnostics R99) - OR - DNA/RNA Shield ™ Swab Collection Kit (Cat. No. R1107-E from Zymo Research, 1 mL. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.
5. Aspirate and lavage samples should be collected according to facility protocols. See SPECIMEN COLLECTION AND HANDLING below.
6. 100% Isopropanol
7. 95% - 100% Ethanol
8. 70% Ethanol
9. Beta-mercaptoethanol (BME)
10. Zymo Research Quick-DNA/RNA Viral MagBead Kit, Cat. Nos. R2140, R2141 R2140-E, R2141-E, or *Quick*-RNA Viral Kit, Cat. No. D7020 or D7021.
11. KingFisher™ Flex Purification System from ThermoFisher Scientific, Cat. No. 5400630-KF
12. Mini microcentrifuge (6,000RPM) for 1.5mL tubes (Heathrow Scientific HS120301 or equivalent)
13. Benchtop vortex mixer
14. Adjustable pipettes (with maximum capacity of 2µL, 10µL, 20µL, 100µL, 200µL, and 1mL respectively)
15. Disposable DNase/RNase free pipette tips with filters (with capacity of 2µL, 10µL, 20µL, 100µL, 200µL, and 1mL respectively)
16. A Rainin single channel 2-20µL Pipet-Lite XLS+ pipette and LTS LiteTouch tips with filters (20µL capacity) is recommended for loading the XDive™ Capillary Reaction Tubes. Alternatively, an Eppendorf 20µL or 100µL pipette with 0.57mm round gel tips with filters can also be used
17. 1.5mL DNase/RNase free centrifuge tube with cap
18. Molecular/PCR grade water
19. Disposable powder-free gloves

NOTE: Please ensure that all instruments and measurement tools have been installed, calibrated, checked, and maintained according to the manufacturer's instructions and recommendations

WARNINGS AND PRECAUTIONS

Carefully read these instructions before starting the procedure.

1. For *In Vitro* Diagnostic Use.
2. For use under Emergency Use Authorization (EUA) Only.
3. For Prescription Use Only.
4. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

5. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
6. This emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.
7. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.
8. The use of this kit should be conducted by medical professionals, biological professionals, or technicians with proper technical training.
9. Use of this product is limited to personnel specifically instructed and trained to work in the bio-safety lab. Wear personal protective equipment such as disposable powder-free gloves, laboratory coats, and safety goggles when handling specimens and kit reagents.
10. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html. Biological samples and materials that have been in contact with the product should be treated as an infectious biohazard. Exposure to skin and mucosa should be prevented. Related local regulations should be followed for the disposal of the materials.
11. All kit components and testing protocols are prepared and validated only for their intended purpose. Replacement or modification of any of the contents will affect the testing performance of the kit.
12. Do not use components of the kit after the expiration date. Components from different lot of the kit should not be used interchangeably.
13. Once a frozen component within the kit is thawed, it is suggested to use them up within one operation based on examination demand. If the remaining components are not used immediately, they should be restored at -20°C.
14. The viral RNA and RT-PCR premix are sensitive to temperature. Once the sample RNA and RT-PCR premix are taken out of the -20°C freezer, keep them on an ice or cooling block and prepare the master mix on an ice or cooling block as well.
15. **Caution**: Avoid microbial and DNase/RNase contamination of the specimen and the kit components.
16. **Caution**: Primer and Probe Mix should be kept out of light.
17. **Caution**: Always use DNase/RNase-free disposable aerosol-blocking pipette tips.
18. **Caution**: Store positive and/or potentially positive material separately from all other kit components.
19. **Caution**: Positive control material may degrade due to repeated freezing, thawing, and pipetting. It is recommended to aliquot the positive control into 10µL amounts in DNase/RNase-free tubes for individual use.
20. **Caution**: Do not open the Capillary Reaction Tubes after amplification to avoid cross contamination with amplicons.
21. Additional controls may need be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
22. Discard sample and assay waste according to local safety regulations.

Work Areas

The KingFisher™ Flex Purification System and the XDive™ Superfast Real-Time PCR System instruments should be operated in separate dedicated areas. The use of two dedicated areas (Sample Preparation Area and Amplification Area) within the laboratory is recommended when performing the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0.

The Sample Preparation Area is dedicated to processing samples and adding processed samples and controls to the Capillary Reaction Tubes. All reagents used in the Sample Preparation Area should always remain in this dedicated area. Do not bring amplification product into the Sample Preparation Area.

The Amplification Area is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.

Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, no template control, positive control, or specimens.

Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.

QUALITY CONTROLS

1. A positive control is needed to monitor if the rRT-PCR reaction is working properly. It shall be run along with the samples for every run.
2. A negative control of the No Template Control (NTC) should be run through the entire sample preparation process, from sample extraction to rRT-PCR. It monitors if the whole procedure is contaminated. It shall be run along with the samples for every run.
3. Primers and probes for the RNase P gene are included in the kit primer and probe mix as an **Internal Control** to monitor the quality of sample collection, handling, and validity of the rRT-PCR amplification when negative patient results are obtained. It is used in each sample amplification and will be non-reactive when external controls are run as no RNase P is present.

SPECIMEN COLLECTION AND HANDLING

Sample Type

Anterior nasal, mid-turbinate, nasopharyngeal or oropharyngeal swabs, nasal wash/aspirates and bronchoalveolar lavage (BAL).

Sample Collection Requirements and Procedure

1. The sample collection shall be collected with synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.
2. Viral transport media (VTM) that may be used for sample storage and transport after collection include Hardy Diagnostics Viral Transport Medium (Hardy Diagnostics R99), and the DNA/RNA ShieldTM Swab Collection Kit (Cat. No. R1107-E from Zymo Research, 1 mL),
3. Human upper respiratory specimens collected into DNA/RNA Shield™ Swab Collection Kits from Zymo Research may be used for sample storage and transport after collection.
4. Refer to the instructions of the sample collection device for proper use.
5. Ensure all collection materials are labelled with the correct identifying information.
6. Swabs from the same patient shall be placed into one storage tube in order to increase the viral load.

Sample Transportation and Storage Requirements

1. Specimens in VTM may be stored at ambient temperature (15 - 30°C) up to 48 hours or at -70°C for longer term storage.
2. Specimens in DNA/RNA Shield™ from Zymo Research can be stored at ambient temperature (15 - 30°C) not to exceed 4 days.
3. Detailed information on packing, shipping, and transporting specimens can be found in the Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). For more information, refer to:

   Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.htmll

   Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

ASSAY Protocol

Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 Procedure

The operator should be trained to operate the XDive™ Superfast Real-Time PCR system and, for automated sample preparation, the KingFisher™ Flex Purification System. Operators should have a thorough knowledge of the software run on the respective instruments and must follow good laboratory practices.

XDive™ Superfast Real-Time PCR System requires that the XDive™ Version V2.9.2 software (or higher) be installed prior to performing the assay. Refer to the XDive™ User Manual and the KingFisher™ Flex Purification System manual for a detailed description of how to set up and use the system.

Sample Preparation and RNA Extraction Protocols

A. Manual RNA Extraction Protocol (Refer to the Zymo Research Quick-DNA/RNA™ Viral MagBead instructions for use, Catalog Numbers R2140, R2141)

1. Reagent Preparation:

1.1 Add beta-mercaptoethanol (BME) to the Viral DNA/RNA Buffer to a final dilution of 0.5% (v/v).

Example: 0.5 mL BME per 100 mL **Viral DNA/RNA Buffer** (Zymo Research cat. No. D7020-1-100).

1.2 Reconstitute lyophilized Proteinase K to a concentration of 20 mg/ml prior to use.

Example: Add 210 µL of Proteinase K Storage Buffer to reconstitute the lyophilized 5 mg Proteinase K or add 1,040 µL of Proteinase K Storage Buffer to reconstitute the lyophilized 20 mg Proteinase K. **Store at -20 ± 5 ˚C after use.**

1.3 Add 100% Isopropanol to MagBead DNA/RNA Wash 1 concentrate to a final dilution of 40% (v/v).

Example: 20 ml Isopropanol per 30 ml MagBead **DNA/RNA Wash 1** concentrate (R2130-1-30) or 80 mL of Isopropanol per 120 ml MagBead DNA/RNA Wash 1 concentrate (R2130-1-120).

1.4 Add 100% Isopropanol to MagBead DNA/RNA Wash 2 concentrate to a final dilution of 60% (v/v).

Example: 30 mL Isopropanol per 20 mL MagBead DNA/RNA Wash 2 concentrate (R2130-2-20) or 120 mL of Isopropanol per 80 mL **MagBead DNA/RNA Wash 2** concentrate (R2130-2-80).

2. **Sample Preparation**:

Perform all steps at room temperature (20 ± 5 °C)

Important: The **No Template Contro**l (**NTC**) should be run through the entire sample preparation process, from sample extraction to rRT-PCR.

2.1 Inside the biosafety cabinet, take the DNA/RNA Shield™ Collection Tube out of the biohazard bag. Perform the following tasks outside of the biosafety cabinet:

2.1.1 Calculate and aliquot the amount of **Proteinase K** (from **Step 1.2**) needed for this batch of samples, **including the NTC**. **Example**: 3 µL of **Proteinase K** for the **NTC**, 10 µL of **Proteinase K** for each ml of swab sample, and/or 40 µL of **Proteinase K** for each aspirate or lavage sample. **Store the Proteinase K at -20 ± 5 ˚C.**

2.2 Place the sample tubes on a rack. Spray the outside of the tubes with 70% Ethanol and wait 10 minutes for the tubes to dry.

2.3 Once the tubes are dry, label the cap and/or the side of the tube sequentially starting with the number "1" up to the maximum number of samples. Place tubes back on the rack after labeling.

2.4 Hold the top of tube and flick downward to pull the liquid to the bottom of the tube.

2.5 If processing a swab sample, follow these instructions:

2.5.1 Unscrew the tube cap to open the tube. Add 10 µL Proteinase K (aliquoted from Step 2.2.1) per 1 mL sample.

2.5.2 Close and cap the tube securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.5.3 Incubate the sample mixture at room temperature for 15 minutes.

2.6 **If processing an aspirate or BAL sample, follow these instructions:**

2.6.1 Unscrew the tube cap to open the tube. Add 40 µL Proteinase K (aliquoted from Step 2.2.1) to the sample.

2.6.2 Close and cap the tube securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.6.3 Incubate the sample mixture at room temperature for 15 minutes.

2.6.4 Add an equal volume of DNA/RNA Shield™ (2X concentrate, Cat No R1200-25 and R1200-125) to a volume of liquid sample (1:1) and mix well.

2.7 For the No Template Control (NTC):

2.7.1 Transfer 300 µL of **NTC** into a new 1.5 mL microcentrifuge tube and label the microcentrifuge tube appropriately. Add 3 µL Proteinase K (aliquoted from Step 2.2.1) to the NTC.

2.7.2 Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.7.3 Incubate the NTC mixture at room temperature for 15 minutes.

2.8 After incubation, hold the top of tube and flick downward to pull the liquid to the bottom of the tube.

2.9 Transfer 300 µL of the Proteinase K-treated sample into a new 1.5 mL microcentrifuge tube and label the microcentrifuge tube with the sample number from Step 2.4.

2.10 Add 600 µL Viral DNA/RNA Buffer (from Step 1.1) to the NTC and sample microcentrifuge tubes.

2.11 Vortex the bottle of MagBinding Beads for 15 seconds at max speed.

2.12 Add 20 µL MagBinding Beads to the sample and NTC. After every 5 samples, cap and re-vortex

the bottle containing MagBinding Beads for 15 seconds to keep the beads in suspension.

2.13 Bring the capped microcentrifuge tube outside the biosafety cabinet. Place the tube onto the microcentrifuge tube carousel. Turn-on the BioShake for 10 minutes at 1,800 RPM at room temperature (20 ± 5 °C) to keep the MagBinding Beads suspended in solution.

2.14 Take the microcentrifuge tube off the BioShake. Place the tubes into the benchtop microcentrifuge. Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid.

2.15 Place the microcentrifuge tube on a magnetic rack and incubate for 30 seconds to pellet the MagBinding Beads. Open the flip-cap. Hold the bottom of the tube while opening to secure the tube and avoid splashing.

2.16 Without touching the MagBinding Beads pellet, slowly aspirate and discard the clear supernatant into a liquid waste bottle.

2.17 Add 500 µl MagBead DNA/RNA Wash 1 (from Step 1.3) to the microcentrifuge tube.

2.18 Vortex for 10 seconds at max speed or shake using the BioShake for 30 seconds at 1,800 RPM. Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid.

2.19 Place the tube onto a magnetic rack and incubate for 1 minute to pellet the MagBinding Beads. Open the flip-cap. Hold the bottom of the tube while opening to secure the tube and avoid splashing.

2.20 Without touching the MagBinding Beads pellet, slowly aspirate and discard the clear supernatant.

2.21 Repeat Step 2.18 – 2.21 using the buffers below in place of MagBead DNA/RNA Wash 1. Samples should be transferred to a new 1.5 ml microcentrifuge tube after resuspension with the final Ethanol wash in Step 2.22.2.

2.21.1. 500 µL MagBead DNA/RNA Wash 2 (from Step 1.4)

2.21.2. 900 µL Ethanol (95-100%)

2.21.3. 500 µL Ethanol (95-100%)

2.22 After removing the Ethanol supernatant (from Step 2.21.3), use a 10 µL pipette to completely aspirate any residual Ethanol.

2.23 Keep the flip-cap open and air dry the MagBinding Beads for 15 minutes on a microcentrifuge rack.

2.24 Add 50 µL DNase/RNase-Free Water to the MagBinding Beads. Mix well by vortexing for 30 seconds at max speed. Close the flip-cap.

2.25 Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid. Then vortex again at Speed 6 for 15 seconds. Hold the tube firmly while vortexing to avoid having the MagBinding Beads splash onto the flip-cap.

2.26 Incubate for 10 minutes at room temperature on a microcentrifuge rack.

2.27 Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid.

2.28 Transfer the tube to a magnetic rack. Incubate for 30 seconds to pellet the MagBinding Beads.

2.29 Tilt the magnetic rack 45 degrees to allow the magnetic beads to move up the microcentrifuge tube wall for easy eluate aspiration.

2.30 Without touching the MagBinding Bead pellet, aspirate as much of the eluate as possible and transfer to a new Hard-Shell 96-Well PCR plate.

2.31 Using a sealing film seal the Hard-Shell 96-Well PCR plate containing the purified RNA samples.

Note: The samples can be immediately used to set up the rRT-PCR Capillary Reaction Tubes in the Sample Preparation Area or stored at ≤ -70 °C.

B. Spin Column RNA Extraction Protocol (Refer to the Zymo Research Quick-DNA/RNA™ Viral Kit instructions for use, Catalog Numbers D7020 and D7021)

1. **Buffer Preparation:**

   1.1. Add beta-mercaptoethanol (BME) to the Viral DNA/RNA Buffer to a final dilution of 0.5% (v/v).

   Example: 0.5 mL BME per 100 mL Viral DNA/RNA Buffer (D7020-1-100).

   1.2. Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml Viral Wash Buffer concentrate (D7020) or 96 ml of 100% ethanol (104 ml of 95% ethanol) to the 24 ml Viral Wash Buffer concentrate (D7021).

2. **Sample Preparation**
   Perform all steps at room temperature (20-30°C). Up to 400 µL sample can be processed per prep.

   2.1. Swabs (VTM): Proceed directly with purification, below.

   2.2. Liquids (aspirates, BAL): Add an equal volume of DNA/RNA Shield™ (2X concentrate, Cat No R1200-25 and R1200-125) to a volume of liquid sample (1:1) and mix well. Proceed with purification, below.

   2.3. Samples in DNA/RNA Shield™ collection devices (swabs, aspirates or BAL): Proceed directly with purification, below.

3. **DNA/RNA Purification**
   Perform all steps at room temperature and centrifugation at 10,000-16,000 x g. The sample input can be scaled up or down, proportionally.

   3.1. Add 800 µL Viral DNA/RNA Buffer to each 400 µL sample1 (2:1) and mix well.

   3.2. Transfer the mixture into a Zymo-Spin™ IIC-XLR Column2 in a Collection Tube and centrifuge for 2 minutes. Transfer the column into a new collection tube.

   3.3. Add 500 µL Viral Wash Buffer to the column, centrifuge for 30 seconds and discard the flow-through. Repeat this step.

   3.4. Add 500 µL ethanol (95-100%) to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube (not provided).

   3.5. Add 50 µL DNase/Rnase-Free Water directly to the column matrix and centrifuge for 30 seconds.

   3.6. See the Zymo Research Quick-DNA/RNA™ Viral Kit instructions for use for additional sample treatment options

C. Automated RNA Extraction Protocol

1. **Buffer Preparation:**

   1.1. Add beta-mercaptoethanol (BME) to the Viral DNA/RNA Buffer to a final dilution of 0.5% (v/v).

   Example: 0.5 mL BME per 100 mL **Viral DNA/RNA Buffer** (D7020-1-100).

   1.2. Vortex the bottle containing MagBinding Beads for 30 seconds at max speed. Then add MagBinding Beads to the Viral DNA/RNA Buffer (containing BME from Step 1.1) to a final dilution of 3.3% (v/v).

   Example: 3.4 mL **MagBinding Beads** per 100 mL **Viral DNA/RNA Buffer**.

   1.3. Reconstitute lyophilized Proteinase K to a concentration of 20 mg/mL prior to use.

   Example: Add 210 µL of **Proteinase K Storage Buffer** to reconstitute the lyophilized 5 mg **Proteinase K** or add 1,040 µL of **Proteinase K Storage Buffer** to reconstitute the lyophilized 20

mg Proteinase K. **Store at -20 ± 5 ˚C after use.**

1.4. Add 100% Isopropanol to MagBead DNA/RNA Wash 1 concentrate to a final dilution of 40% (v/v).

Example: 20 mL Isopropanol per 30 mL **MagBead DNA/RNA Wash 1** concentrate (R2130-1-30) or 80 mL of Isopropanol per 120 mL **MagBead DNA/RNA Wash 1** concentrate (R2130-1-120).

1.5. Add 1**00%** Isopropanol to MagBead DNA/RNA Wash 2 concentrate to a final dilution of 60% (v/v).

Example: 30 mL Isopropanol per 20 mL **MagBead DNA/RNA Wash 2** concentrate (R2130-2-20) or 120 mL of Isopropanol per 80 mL **MagBead DNA/RNA Wash 2** concentrate (R2130-2-80).

2. Sample Preparation:

Perform all steps at room temperature (20 ± 5 °C)

Important: The **No Template Contro**l (**NTC**) should be run through the entire sample preparation process, from sample extraction to rRT-PCR.

2.1. Inside the biosafety cabinet, take the DNA/RNA Shield™ Collection Tube out of the biohazard bag.

2.2. Perform the following tasks outside of the biosafety cabinet:

2.2.1. Calculate and aliquot the amount of Proteinase K (from Step 1.3) needed for this batch of samples. Example: 3 µL of Proteinase K for the NTC or 10 µL of Proteinase K for each mL swab sample. Store the Proteinase K stock at -20 ± 5 °C.

2.3. Place the sample tubes on a rack. Spray the outside of the tubes with 70% Ethanol and wait 10 minutes for the tubes to dry.

2.4. Once the tubes are dry, label the cap and/or the side of the tube sequentially starting with the number “1” up to the maximum number of samples. Place tubes back on the rack after labeling.

2.5. Hold the top of tube and flick the tube to pull-down the liquid inside.

2.6. If processing a swab sample, follow these instructions:

2.6.1. Unscrew the tube cap to open the tube. Add 10 µL Proteinase K (aliquoted from Step 2.2.1) per mL of sample.

2.6.2. Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.6.3. Incubate the sample mixture at room temperature for 15 minutes.

2.7. Liquids (aspirates, BAL): Add an equal volume of DNA/RNA Shield™ (2X concentrate, Cat No R1200-25 and R1200-125) to a volume of liquid sample (1:1) and mix well. Proceed with purification, below.

2.8. For the No Template Control (NTC):

2.8.1. Transfer 300 µL of NTC into each of two (2) new 1.5 mL microcentrifuge tube and label the microcentrifuge tube appropriately. Add 3 µL Proteinase K (aliquoted from Step 2.2.1) to each NTC.

2.8.2. Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.8.3. Incubate the NTC mixture at room temperature for 15 minutes.

3. KingFisher™ Plates Preparation:

3.1. Prepare all KingFisher™ Plates outside of the biosafety cabinet.

3.2. Sample Plate Preparation:

3.2.1. Vortex the container of Viral DNA/RNA Buffer (containing BME and MagBinding Beads from Step 1.2) for 10 seconds at maximum speed. Aliquot 620 µL into a new KingFisher™ Deepwell 96 Plate, V-bottom. Fill the well-positions up to the number of samples being processed (maximum 92). Below is a typical sample plate schematic:

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	S7	S15	S23	S31	S39	X	S53	S61	S69	S77	S85
B	NTC	S8	S16	S24	S32	S40	NTC	S54	S62	S70	S78	S86
C	S1	S9	S17	S25	S33	S41	S47	S55	S63	S71	S79	S87
D	S2	S10	S18	S26	S34	S42	S48	S56	S64	S72	S80	S88
E	S3	S11	S19	S27	S35	S43	S49	S57	S65	S73	S81	S89
F	S4	S12	S20	S28	S36	S44	S50	S58	S66	S74	S82	S90
G	S5	S13	S21	S29	S37	S45	S51	S59	S67	S75	S83	S91
H	S6	S14	S22	S30	S38	S46	S52	S60	S68	S76	S84	S92

X = leave empty, NTC = No Template Control, S1 = Sample 1

3.2.2. Label the side of the deepwell plate “Sample Plate”

3.3. Wash 1 Plate Preparation:

3.3.1. Aliquot 500 µL of MagBead DNA/RNA Wash 1 (containing Isopropanol from Step 1.4) into a new KingFisher™ Deepwell 96 Plate, V bottom. Fill the well positions according to the diagram on Step 3.2.1.

3.3.2. Label the side of the deepwell plate “Wash 1”.

3.4. Wash 2 Plate Preparation:

3.4.1. Aliquot 500 µL of MagBead DNA/RNA Wash 2 (containing Isopropanol from Step 1.5) into a new KingFisher™ Deepwell 96 Plate, V bottom. Fill the well positions according to the diagram on Step 3.2.1.

3.4.2. Label the side of the deepwell plate “Wash 2”.

3.5. EtOH 1 Plate Preparation:

3.5.1. Aliquot 900 µL of 95% Ethanol into a new KingFisher™ Deepwell 96 Plate, V bottom. Fill the well positions according to the diagram on Step 3.2.1.

3.5.2. Label the side of the deepwell plate “EtOH 1”.

3.6. EtOH 2 Tip Plate Preparation:

3.6.1. Add 500 µL of 95% Ethanol into a new KingFisher™ Deepwell 96 Plate, V bottom. Fill the well positions according to the diagram on Step 3.2.1.

3.6.2. Slowly load the KingFisher™ 96 tip comb into the plate. Do not cause the Ethanol to splash out of the well.

3.6.3. Label the side of the deepwell plate “EtOH 2 Tip”.

3.7. Elution Plate Preparation:

3.7.1. Add 60 µL of Dnase/Rnase-Free Water into a new KingFisher™ 96 KF microplate. Fill the well positions according to the diagram on Step 3.2.1.

3.7.2. Label the side of the plate “Elution”.

4. **Transferring Samples into the Sample Plate:**

4.1. Bring the prepared Sample Plate (from Step 3.2) into the biosafety cabinet.

4.2. Inside the biosafety cabinet, hold the sample tube from the top and flick to pull- down the liquid.

4.3. Transfer 300 µL of Proteinase K-treated sample in DNA/RNA Shield™ or VTM (from Step 2.6 or Step 2.7) and NTC (from Step 2.8) into the Sample Plate following the plate diagram in Step 3.2.1. Prevent cross contamination by dispensing the sample after the pipette tip is half-way into the well. DO NOT let liquid from the pipette tip touch another well while entering or removing the tip from the well.

4.4. After all the samples have been transferred bring the Sample Plate outside of the biosafety cabinet.

5. **Operating the KingFisher™ Flex and Loading Plates:**

5.1. Ensure the KingFisher™ Flex machine is on.

5.2. Open the plastic shield sliding door.

5.3. Press the “Right” arrow on the machine to highlight the center icon. The menu highlight will turn ORANGE.

5.4. Press the “Down” arrow to highlight the “RNA” menu then press “OK”.

5.5. Highlight the “SARS-CoV-2 RNA V7” program. Ensure the SARS-CoV-2 RNA V7 program is downloaded from the Zymo Research Website.

5.6. Press the green “Start” button to initiate the program. The screen will now indicate where to load the KingFisher™ plates on the turntable.

5.7. Load the prepared Elution Plate (from Step 3.7) onto the turntable position “1”. Load the Elution Plate so that the “A1” well is at the A1 position indicated on the turntable. Ensure the plate sits flat on the turntable.

5.8. Press “Start”. The turntable will rotate to an empty position. The screen will indicate which plate (from Step 3.3 to 3.6) to load next and onto which turntable position. Load the plate in a similar manner to Step 5.7. Repeat until all the plates have been loaded onto the deck. The Sample Plate is the last plate to be loaded.

5.9. Once the Sample plate is loaded, close plastic shield door and press "Start" to begin the RNA extraction program.

5.10. The program takes about 50 minutes to complete.

5.11. After program is complete, the screen will ask to remove the plates from the turntable.

5.12. Open the plastic shield sliding door and carefully remove the Elution Plate from the turntable.

5.13. Place the Elution Plate on the bench away from the KingFisher™ Flex and seal the plate to prevent contamination. If not used immediately, then store the RNA samples at ≤ -70°C.

5.14. Press “Start” to rotate the turntable and remove the remaining plates one-by-one.

5.15. Discard liquid from the Sample Plate and remaining KingFisher™ plates according to institutional practices.

Note: The Elution plate from Step 5.15 now contains the **purified RNA samples** to be run through the rRT-PCR assay. The samples can be immediately used to set up the Capillary Reaction Tubes in the Sample Preparation Area or stored at ≤ -70 °C.

Amplification Reaction Set-up Procedure

The assay can be used with purified RNA extracted from the automated extraction protocol above. Purified RNA samples are directly mixed with the prepared Master Mix below and analyzed using the XDive™ Superfast Real-Time PCR System. Results are ready to be interpreted after the reverse transcription and real time PCR steps are completed.

rRT-PCR Reaction Setup

Steps of rRT-PCR Reaction Setup are to be performed in the 'Sample Preparation Area'

Prepare Positive/Negative Control

1. The Positive Control is *in vitro* transcribed COVID-19 gene fragment and may degrade over time due to repeated freezing, thawing, and pipetting. It is recommended to aliquot the positive control stock into 10µL volumes in DNase/RNase-free tubes for individual use.
2. The negative control of the No Template Control (NTC) will be run through the entire sample RNA extraction process and is ready for rRT-PCR.

Prepare RT-PCR Master Mix

1. Take out the reagent tubes from the kit package and thaw in room temperature. Each test run on the XDive™ Superfast Real-Time PCR system can accommodate up to 16 samples, which includes a positive and a negative control.
2. Use Table 2 below to calculate the total amount of each reagent for the master mix. Add 10% more volume to account for pipetting error. Prepare larger volumes of master mix as necessary according to the total number of samples and controls needing to be tested through the day:

Table 2. Master Mix Volumes Required

Reagent	1x Volume (µL)	1.1xN Volume (µL)	Daily Use Volume (µL)
COVID-19 Primer and Probe Mix	3	3.3xN	
Superfast RT-PCR Enzyme Mix	7	7.7xN	
Total	10	11xN	

Warning! The prepared master mix must be used within the same day. Leftover master mix must be discarded after 24 hours.

3. Pipette all the reagents into a pre-labelled 1.5mL RNase free tube. Pause vortex the tube for 5 seconds at 3000rpm. Store the tube on an ice or cold block.

Load controls and samples

1. For each testing run, acquire the appropriate number of 0.5mL RNase free tubes with caps. Label the tubes with the proper sample information and a sample number from 1 to 16 including positive and negative controls. Add 10µL of RT-PCR Master Mix into each tube.
2. Add 10µL of extracted sample, positive control, or negative control into the appropriate tube according to the sample name on the tube label and vortex the mixes for 5 seconds.
3. Put the XDive™ PCR Capillary Reaction Tubes holder onto the "Fill" side of the tube loading/releasing stand, with the holder handle close to the user.
4. Gather the appropriate number of the XDive™ PCR Capillary Reaction Tubes with caps. Mark the capillaries on the cap with the appropriate sample numbers from 1 to 16. Pipette the total 20µL of sample reaction mix (the solution of master mix and extracted sample or control) into the matching Capillary Reaction Tube using a sharp pipette tip (e.g. Rainin LTS 20µL, PN 30389226), place the tube on the

Capillary Tube Holder, and then close the lid of the tube. The protruding link connecting the Capillary Reaction Tube and its cap should be on the same side as the handle on Capillary Reaction Tube holder.

5. Load the filled Capillary Reaction Tube holder onto the provided mini centrifuge with adaptor. Use another empty Capillary Reaction Tube Holder on the opposite side of the adaptor as balance and spin the capillaries at 1500rpm for 5 seconds to pull the liquid to the bottom and remove any air bubbles that may have been generated at the loading step.
6. Bring the Capillary Reaction Tubers with the holder to the XDive™ system located in the '**Amplification Area**'. Push open the instrument front door. Load the entire Reaction Capillary Tube Holder onto the instrument following the instructions in the XDive™ User Manual. Close the instrument door and the samples are ready to run.

Real-Time PCR Machine Setup

Steps of '**Real-Time PCR Machine Setup**' are to be performed in the '**Amplification Area**'

rRT-PCR Thermal Condition

Use the following thermal cycling conditions to amplify the samples on the XDive™ Superfast Real-Time PCR instrument (Table 3).

Table 3. XDive™ Thermocycler Settings

PCR Step	Reverse Transcription	Hot Start	45 Cycles	
			Denaturation	Annealing/Extension
Temperature (°C)	50	95	93	60
Time (sec)	180	60	0	10

Software Setup

Refer to the XDive™ Superfast Real-Time PCR system User Manual for instructions regarding the operation of the instrument and software setup procedures.

1. Log into the software.
2. Select "Template" tab and choose the 'Hi-Sense™ COVID19 Molecular Testing Kit 1.0' from the directory.
3. Define the experiment name.
4. Review the rRT-PCR thermal condition.
5. Review the target names and dye name for each target. The assay should use FAM for the N1 and N2 genes, SUN for Orf1ab gene, and CY5 for RNase P gene (internal control).
6. Review the reagent information and confirm it is the Hi-Sense™ COVID19 Molecular Testing Kit 1.0.
7. Set up sample names in the sample table. Add new samples with the proper sample information.
8. Select each sample and then assign it with all three targets and the reagent. Verify that the sample number matches the number of the position on the capillary tube holder.
9. Start the run and after the run is complete, click the "Analyze Last Experiment" button.

RESULT INTERPRETATION

Define The Data Analysis Method

It is recommended to use the default value of the Ct detection threshold and baselining in the software. The default Ct detection threshold is a fluorescence value is 15 for the two N gene targets, 15 for Orf1ab target, and 10 for RNase P target respectively. The Ct value of each target for each sample or control will be displayed in the result table. Please refer to the XDive™ User Manual to make the adjustments. The corresponding Ct threshold line is shown in the graph panel.

Quality Control

The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 requires positive control, negative control, and internal control components to monitor sample and reagent quality and the reliability of the results for the entire process. All test control results should be examined prior to interpretation of sample results. Positive control, negative control, and internal control (IC) results should meet the requirements listed in the below Table 4 to ensure valid results. If the controls are not valid, negative patient results cannot be interpreted.

1. Check the Ct values of the positive control, negative control, and the internal control of each sample in the same run and compare to the quality control requirements in the following table.

Table 4. Quality Control Requirements

Sample	N gene (FAM)	Orf1ab gene (SUN)	IC (CY5)	Interpretation
Positive Control (PC)	Ct ≤ 38	Ct ≤ 38	Ct ≤ 38	Reagents are working properly for detecting all targets.
Negative Control (NTC)	Ct > 38 or UND	Ct > 38 or UND	Ct > 38 or UND	The process is free of contamination.

* Note that Ct values for positive control runs are likely around 34 based on the default Ct detection threshold setting. Users may perform independent calibration runs to establish a passing criterion based on their Ct detection threshold settings. UND = undetermined.

2. For Positive Control (PC), if any of the N gene, Orf1ab, and Internal Control (IC) targets is negative (Ct > 38 or UND), the whole reaction run shall be determined to be failed. The user should repeat the run.
3. For Negative Control (NTC), if any of the N gene, Orf1ab, and Internal Control (IC) targets is positive (Ct ≤ 38), an unknown source of sample contamination is suspected. The work area should be cleaned thoroughly, and the sample should then be retested.
4. If both positive and negative control Ct values meet the QC requirements, the run can be determined successful. Move on to the next step to evaluate the sample results.

Determine The Sample Results

1. Check the Ct values of each sample in the same run and compare to the Ct interpretation thresholds in the Table 5 below.

Table 5. Sample Result Ct Interpretation Thresholds

SARS-CoV-2 Target		IC Target	Results	Interpretation
N (FAM)	Orf1ab (SUN)	RNase P (CY5)		
> 38 or UND	> 38 or UND	≤ 38	Negative	Indicates the absence of SARS-CoV-2 RNA
≤ 38	Any Value*	Any Value	Positive	Indicates the presence of SARS-CoV-2 RNA
Any Value*	≤ 38			
> 38 or UND	> 38 or UND	> 38 or UND	Invalid	Inability to conclusively determine presence or absence of SARS-CoV-2 RNA. This may be due to 1) internal control failure; or 2) insufficient specimen volume. The sample needs to be retested.

* In the case of one SARS-CoV-2 target positive (Ct ≤ 38) and one SARS-CoV-2 target negative (Ct > 38 or UND), the result is suggestive of: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in one of the target regions, or 3) other factors. UND = undetermined.

2. If the result for a specimen is SARS-CoV-2 RNA negative, the Ct value of the internal control must be ≤ 38, otherwise the result of that specimen is invalid.
3. If the result for a specimen is SARS-CoV-2 RNA positive, the Ct value of the internal control is not required to be considered valid.
4. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.
5. Visually inspect every amplification curve for each sample that exhibits COVID-19 signal and note any sample displaying aberrant amplification curves. Samples giving aberrant amplification results should be rerun. If the aberrant curve is still observed, contact customer service.

Post-Processing Procedures

1. After the completion of the PCR run, remove the Capillary Reaction Tubes from the XDive™ Superfast Real-Time PCR system and dispose of according to the instrument guide.
2. Place the Capillary Reaction Tubes into a sealable plastic bag and dispose according to the XDive™ Superfast Real-Time PCR system guide.
3. Clean the XDive™ Superfast Real-Time PCR system according to guide recommendations.

LIMITATIONS

1. This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization (EUA) only.
2. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
3. For use only by personnel trained in the techniques of rRT-PCR, and in vitro diagnostic procedures. A thorough understanding of the instructions for use is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following these instructions for use.
4. Performance of the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 has only been established in nasopharyngeal swab specimens. Oropharyngeal, anterior nasal and mid-turbinate swabs, nasal aspirates/washes and BAL are also considered acceptable specimen types for use with this assay, but performance has not been established.

5. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
6. Amplification and detection of SARS-CoV-2 with the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 has only been validated with the XDive™ Superfast Real-Time PCR System and the specified methods described in this document. Use of other instrument systems or methods may cause inaccurate results.
7. False negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay, if the virus has genomic mutations, insertions, deletions, or rearrangements, or if performed very early in the course of illness.
8. False positive results may occur. Repeat testing or testing with a different device may be mandated according to local, state, and/or federal regulations or the guidelines of accrediting organizations.
9. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
10. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
11. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics, or immunosuppressant drugs have not been evaluated. The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
12. This test cannot rule out diseases caused by other bacterial or viral pathogens.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-useauthorizations-medical-devices/in-vitro-diagnostics-euas. To assist laboratories using the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product must include with any result reports of your product all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product must use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to use your product, are not permitted.

C. Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product mustl have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and You (via email: EUAReporting@onsitegene.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labelling.

G. You, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

Limit of Detection

The preliminary LoD range was determined by testing ten-fold dilutions (from 8.33 x 10^3 to 8.3 GCE/mL) of heat-inactivated SARS-CoV-2 (BEI, Cat. No. NR-52286, isolate USA-WA1/2020) prepared in negative nasopharyngeal swab matrix in VTM and tested in replicates of five. Automated extraction was performed using the Zymo *Quick*-DNA/RNA Viral MagBead kit (Catalog Cat. Nos. R2140-E, R2141-E) and the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0, run on the XDive™ Superfast Real Time PCR System (Table 6).

Table 6. Range-Finding LoD Results, Ct Values

Extraction Method	Virus Concentration	N, Mean Ct	Orf1ab, Mean Ct	RNase-P, Mean Ct	Positive Rate	% Reactive
Zymo Quick-DNA/RNA Viral MagBead Kit KingFisher Flex	0 GCE/rxn, 0 GCE/mL	UND	UND	20.50	0/5	0%
	5 GCE/rxn, 83 GCE/mL	38.50	37.70	22.00	4/5	80%
	50 GCE/rxn, 833 GCE/mL	35.36	35.54	21.50	5/5	100%
	500 GCE/rxn, 8,333 GCE/mL	30.38	30.68	20.40	5/5	100%

Twenty replicates were further tested between the lowest concentrations giving 5/5 positive results (833 GCE/mL) and its two-fold dilution (416.5 GCE/mL). The concentration giving ≥ 19/20 results was taken as the LoD, found to be 833 GCE/mL. Confirmatory LoDs were performed after extraction using the automated method (Catalog No R2140-E/R2141-E) in both VTM and DNA/RNA Shield, the manual method (Quick-DNA/RNA™ Viral MagBead kit, Cat. Numbers 2140, R2141) and the spin column method (Quick-DNA/RNA™ Viral Kit Cat. Numbers D7020 and D7021) extraction methods (Table 7).

Table 7. Confirmatory LoD Results, Ct Values

Media	Extraction Method	Virus Concentration	N, Mean Ct	Orf1ab, Mean Ct	RNase-P, Mean Ct	Positive Rate	% Reactive
VTM	Zymo Quick-DNA/RNA Viral MagBead Kit KingFisher Flex	0 GCE/mL, (0 GCE/rxn)	UND	UND	21.60	0/20	0%
		416.5 GCE/mL (25 GCE/rxn)	40.20	39.10	21.90	17/20	85%
		833 GCE/mL (50 GCE/rxn)	36.37	36.17	25.29	20/20	100%
DNA/RNA Shield	Zymo Quick-DNA/RNA Viral MagBead Kit KingFisher Flex	833 GCE/mL (50 GCE/rxn)	34.93	35.18	23.56	20/20	100%
VTM	Zymo Quick-DNA/RNA Viral MagBead Kit Manual	833 GCE/mL (50 GCE/rxn)	34.99	34.96	25.04	20/20	100%
VTM	Quick-DNA/RNA Viral Kit Spin Column	833 GCE/mL (50 GCE/rxn)	32.84	32.66	23.64	19/20	95%

Inclusivity (analytical reactivity)

In silico analysis was used to determine the extent of homology between the primer and probe sequences included in the test kit and the sequences of SARS-CoV-2 isolates available in public databases (NCBI Genbank and GISAID). *In silico* analysis were performed using ROSALIND DxM software.

The N and Orf1ab gene primer and probe sequences included in the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 were entered into the ROSALIND DxM system and compared against the 3,887,496 US GISAID sequences available on the ROSALIND DxM system, which included sequences up to the most recent update on October 17, 2022. None of the incidents are expected to cause a loss in the ability of the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 to detect SARS-CoV-2, as the redundance of N gene and Orf1ab targets mitigates against loss of sensitivity for the mismatches found. Incidents reported by ROSALIND DxM software are shown in Table 8.

Table 8. ROSALIND DxM Incidents in full US database as of October 17, 2022

ROSALIND Incident ID	Affected primer / probe	Severity of Risk	Mismatch frequency			
			Full US database	Previous 30 days	Previous 60 days	Previous 90 days
INC20220625-05317	N1 Probe	5	0.10%	3.07%	1.12%	0.68%
INC20220625-05531	N1 Probe	5	0.26%	3.13%	3.20%	3.10%
INC20220625-05641	N1 Probe	3	3.73%	49.93%	47.53%	44.98%
INC20220625-03535	N2 Probe	2	0.17%	0.26%	0.20%	0.17%
INC20220625-05190	Orf1ab Probe	2	0.02%	0.39%	0.26%	0.22%
INC20220625-05341	N1 Probe	2	37.93%	41.84%	45.80%	48.95%
INC20220625-05537	N1 Probe	2	0.09%	0.10%	0.24%	0.14%
INC20220625-03534	N2 F	0	0.14%	0.11%	0.11%	0.11%
INC20220625-03802	N2 F	0	0.22%	0.18%	0.12%	0.10%
INC20220627-02147	N1 R	0	0.01%	0.23%	0.17%	0.14%
INC20220625-03930	N2 R	0	0.02%	0.33%	0.19%	0.15%

Cross-reactivity (Analytical Specificity)

Potential cross-reactivity was assessed using the NCBI Basic Alignment Search Tool (BLAST) to identify high (>80%) regions of homology between the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 primers and probes and the genomes of potentially cross-reactive microorganisms (Table 9a and 9b).

Table 9a. *In Silico* Cross Reactivity

Microorganisms from the Same Genetic Family	High Priority Microorganisms	
Human coronavirus 229E	Human adenovirus 1	*Bordetella pertussis*
Human coronavirus OC43	Human metapneumovirus	*Candida albicans*
Human coronavirus HKU1	Human respirovirus 1	*Chlamydia pneumoniae*
Human coronavirus NL63	Human respirovirus 2	*Haemophilus influenzae*
SARS coronavirus†	Human respirovirus 3	*Legionella pneumophila*†
MERS coronavirus	Human respirovirus 4	*Mycobacterium tuberculosis*†
	Influenza A virus (H1N1)	*Mycoplasma pneumoniae*
	Influenza B virus	*Pseudomonas aeruginosa*
	Enterovirus D68	*Staphylococcus epidermis*
	Respiratory syncytial virus	*Streptococcus pneumoniae*
	Rhinovirus A	*Streptococcus pyogenes*
		Streptococcus salivarius

Table 9a. †Homology ≥ 80% was found for three organisms, which required additional wet testing.

Table 9b. Organisms with ≥80% *in silico* cross-reactivity

Pathogen	Strain	GenBank Accession #	% N1 Homology			% N2 Homology			% Orf1ab Homology			% RNase P Homology		
			F	R	P	F	R	P	F	R	P	F	R	P
SARS coronavirus	SARS	NC_004718.3	40	92	92	100	72	43	96	57	84	47	45	39
Legionella pneumophila	NCTC12273	NZ_LR134380.1	65	54	67	80	61	57	54	57	48	58	70	52
Mycobacterium tuberculosis	H37Rv	NC_018143.2	60	50	50	80	72	61	50	57	56	63	60	61

Table 9b. Results for three organisms with cross-reactivity ≥ 80%. F=forward primer, R=reverse primer, P=probe.

Wet analysis was performed by adding the potentially interfering pathogens to pooled negative nasopharyngeal matrix (PNM) with and without heat-inactivated SARS-CoV-2 (BEI, Cat. No. NR-52286) at 3X LoD. None of the microorganisms were found to be cross-reactive at the concentrations tested (Table 10).

Table 10. Wet Analysis of Cross-Reactivity

Microorganism Added	Final Concentration	SARS-CoV-2 concentration	Positive Results
None	0	0X LoD	0/3
		3X LoD	3/3
SARS-CoV-1	1.0 E5 PFU/mL	0X LoD	0/3
		3X LoD	3/3
Legionella pneumophila	1.0 E6 CFU/mL	0X LoD	0/3
		3X LoD	3/3
Mycobacterium tuberculosis	1.0 E6 CFU/mL	0X LoD	0/3
		3X LoD	3/3
Pneumocystis jiroveci†	1.0 E6 CFU/mL	0X LoD	0/3
		3X LoD	3/3

†There was no complete genome available to perform *in silico* analysis for *Pneumocystis jiroveci*, so it was included in the wet testing.

Interfering Substance Studies

Substances that may be present in respiratory specimens were evaluated for interference (false negative and false positive results) with the assay. Contrived low positive samples were prepared by adding heat-inactivated SARS-CoV-2 (BEI, Cat. No. NR-52286) at 3X LoD (2500 GCE/ml) into PNM. Interfering substances were added at the indicated concentration (see Table 11) to the low positive and negative specimens, which were each extracted and tested in triplicate.

Table 11. Effect of Potentially Interfering Substances

Substance	Final Substance Concentration	SARS-CoV-2 concentration	Positive Results
Nasal Spray (Afrin Original)	15% v/v	0X LoD	0/3
		3X LoD	3/3
Sore throat and cough lozenges, (Cepacol)	3 mg/mL	0X LoD	0/3
		3X LoD	3/3
Chloroseptic Sore throat spray	5%v/v	0X LoD	0/3
		3X LoD	3/3
Mouth wash	5% v/v	0X LoD	0/3
		3X LoD	3/3
Cough syrup (Robitussin)	5% v/v	0X LoD	0/3
		3X LoD	3/3
Mucin: bovine submaxillary gland, type I-S	2.5 mg/mL	0X LoD	0/3
		3X LoD	3/3
Nicotine or Tobacco	0.03 mg/mL	0X LoD	0/3
		3X LoD	3/3
Toothpaste	0.5% v/v	0X LoD	0/3
		3X LoD	3/3

Carryover and Cross-Contamination

A study was conducted to determine if placing samples with high viral loads adjacent to negative samples could result in cross contamination during the rRT-PCR steps in the XDive Superfast Real-Time PCR System. Negative (nuclease-free water) and 100,000X LoD (8.33 X 10^7 GCE/mL) of transcribed viral RNA samples were placed in adjacent positions and tested. The procedure was repeated 3 times. Every negative sample gave a negative result (UND) and every spiked sample gave a positive result. The results confirmed that there is no carryover of viral particles from strong positive to negative samples during preparation or during amplification in adjacent positions in the XDive Superfast Real Time PCR system (Table 12).

Table 12, Sample Carryover and Contamination Results

Run Number	Tube Position	Sample	N (FAM)	Orf1ab (SUN)	RP (Cy5)	Interpretation
Run 1	P01	NTC	UND	UND	UND	Negative
	P02	10^5X LoD	13.2	13.0	13.7	Positive
	P03	NTC	UND	UND	UND	Negative
	P04	10^5X LoD	12.9	13.1	13.5	Positive
	P05	NTC	UND	UND	UND	Negative
	P06	10^5X LoD	13.0	13.1	13.4	Positive
	P07	NTC	UND	UND	UND	Negative
	P08	10^5X LoD	12.9	13.3	13.5	Positive
	P09	NTC	UND	UND	UND	Negative
	P10	10^5X LoD	12.5	13.3	13.9	Positive
	P11	NTC	UND	UND	UND	Negative
	P12	10^5X LoD	12.9	13.3	13.8	Positive
	P13	NTC	UND	UND	UND	Negative
	P14	10^5X LoD	13.2	13.2	14.0	Positive
	P15	NTC	UND	UND	UND	Negative
	P16	10^5X LoD	13.6	13.5	14.4	Positive
Run 2	P01	NTC	UND	UND	UND	Negative
	P02	10^5X LoD	13.2	13.7	14.0	Positive
	P03	NTC	UND	UND	UND	Negative
	P04	10^5X LoD	12.5	13.1	13.7	Positive
	P05	NTC	UND	UND	UND	Negative
	P06	10^5X LoD	12.6	13.2	13.5	Positive
	P07	NTC	UND	UND	UND	Negative
	P08	10^5X LoD	12.9	13.2	13.6	Positive
	P09	NTC	UND	UND	UND	Negative
	P10	10^5X LoD	12.7	13.1	13.8	Positive
	P11	NTC	UND	UND	UND	Negative
	P12	10^5X LoD	12.8	13.3	14.0	Positive
	P13	NTC	UND	UND	UND	Negative
	P14	10^5X LoD	13.3	13.4	14.1	Positive
	P15	NTC	UND	UND	UND	Negative
	P16	10^5X LoD	14.0	13.7	14.2	Positive
Run 3	P01	NTC	UND	UND	UND	Negative
	P02	10^5X LoD	13.1	13.0	13.8	Positive
	P03	NTC	UND	UND	UND	Negative
	P04	10^5X LoD	12.8	13.1	13.5	Positive
	P05	NTC	UND	UND	UND	Negative
	P06	10^5X LoD	13.2	13.2	13.6	Positive
	P07	NTC	UND	UND	UND	Negative
	P08	10^5X LoD	13.2	13.1	13.8	Positive
	P09	NTC	UND	UND	UND	Negative
	P10	10^5X LoD	14.3	14.4	14.5	Positive
	P11	NTC	UND	UND	UND	Negative
	P12	10^5X LoD	13.4	13.4	14.1	Positive
	P13	NTC	UND	UND	UND	Negative
	P14	10^5X LoD	13.7	13.7	14.2	Positive
	P15	NTC	UND	UND	UND	Negative
	P16	10^5X LoD	14.0	13.8	14.2	Positive

Clinical Evaluation for Patients Suspected of COVID-19

A clinical study was conducted to demonstrate the performance of the Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 on nasopharyngeal swab specimens collected from patients suspected of COVID-19. A total of 130 prospectively collected leftover nasopharyngeal swab media samples were tested including 68 frozen VTM samples and 62 fresh DNA/RNA Shield (Zymo Research) samples. The VTM specimens were collected from a population of 32 male, 19 female and 17 patients with unknown gender, and were 2 to 88 years of age. An FDA authorized high sensitivity RT-PCR test was used as the comparator method. Approximately 21% of the samples were near the LoD of the comparator. The results are shown in the Table 13 below:

Table 13. Clinical Nasopharyngeal Swab Samples Collected in VTM

	Comparator Results		
Hi-Sense COVID-19 Molecular Testing Kit 1.0 Results	**Positive**	**Negative**	**Total**
Positive	38	1	39
Negative	0	29	29
Total	38	30	68

PPA = 38/38 = 100.0% (95% CI = 90.8% - 100.0%)
NPA = 29/30 = 96.7% (95% CI = 83.3% - 99.4%)

The nasopharyngeal swabs in DNA/RNA Shield specimens were collected from a population of 33 male and 29 female patients and were 7 to 99 years of age. An FDA authorized high sensitivity RT-PCR test was used as the comparator method. Approximately 19% of the samples were near the LoD of the comparator. The results are shown in Table 14 below:

Table 14. Clinical Nasopharyngeal Swab Samples Collected in DNA/RNA Shield

	Comparator Results		
Hi-Sense COVID-19 Molecular Testing Kit 1.0 Results	**Positive**	**Negative**	**Total**
Positive	32	0	32
Negative	0	30	30
Total	32	30	62

PPA = 32/32 = 100.0% (95% CI = 89.3% - 100.0%)
NPA = 30/30 = 100.0% (95% CI = 88.7% - 100.0%)

REFERENCES

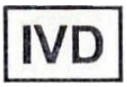
1. CDC Guidelines for clinical specimen handling: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

2. YF Men, Y Fu, ZT Chen, P.A. Sims, W J Greenleaf and YY Hung. Digital polymerase chain reaction in an array of femtoliter polydimethylsiloxane microreactors. Anal. Chem. 2011, 83, 21, 8158-816.

3. Manual of Clinical Microbiology, 11th Edition, Vol. 1, ASM. (2015) pg. 279.

4. EUA Quick SARS-CoV-2 rRT-PCR Kit IFU from Zymo Research: https://www.fda.gov/media/137780/download

SYMBOLS USED

Symbols used on kit label	Remarks
	*In vitro* diagnostic reagent
	Storage temperature range
	Keep out of direct light
	Expiration Date
	Catalogue Number
	Lot Number
	Manufacturer

CONTACT AND SUPPORT

OnsiteGene, Inc.

6373 Nancy Ridge Drive
San Diego, California 92121
TEL: 858-333-5917
Email: enquiry@onsitegene.com
Visit our website at www.onsitegene.com

Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 – KT1010001
Effective Date: 11-2022

Hi-Sense COVID-19 Molecular Testing Kit 1.0

℞ Prescription Use Only IVD For *In Vitro* diagnostic use

For use under Emergency Use Authorization (EUA) only.

This is not the complete Instructions for Use. The complete Instructions for Use may be downloaded at https://www.onsitegene.com/covid-19/.

The Fact Sheet for Healthcare Providers and the Fact Sheet for Patients are also available at https://www.onsitegene.com/covid-19/.

Please contact **OnsiteGene Technical Support** at **1.858.333.5917** or email techsupport@onsitegene.com for questions or if you require a printed copy free of charge or need technical support to access the Instructions for Use.

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This product has been authorized only for the detection of nucleic acid of SARS-CoV-2, not for any other viruses or pathogens;
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb- 3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

XDive™ Superfast Real-Time PCR System User Manual

For *in vitro* Diagnostic use

For use under Emergency Use Authorization (EUA) Only

For Prescription Use Only

Contents

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READ CAREFULLY BEFORE USE.
KEEP FOR FUTURE REFERENCE.

1. Contact Address

Manufacturer

Star-Array Pte Ltd
JTC MedTech Hub
2 Tukang Innovation Grove,
#09-09, Singapore 618305.
Tel: +65-68731788
Website: https://www.star-array.com/
Email Address: enquiry@star-array.com

2. Operating & Storage Condition

Operating Condition

Parameters	Lowest	Highest
Ambient air temperature	16ºC	32ºC
Relative Humidity	20%	60%
Maximum Altitude	1,500m	
Outdoor environment allowed?	No	
Orientation	Upright only (Do not tilt)	

Storage Condition

Parameters	Lowest	Highest
Ambient air temperature	16ºC	32ºC
Relative Humidity	20%	60%
Maximum Altitude	1,500m	
Outdoor environment allowed?	No	
Orientation	Upright only (Do not tilt)	

3. Intended Use

The XDive™ Superfast Real-Time PCR System (XDive), for use with the Hi-Sense COVID-19 Molecular Testing Kit 1.0, performs nucleic acid amplification and detection of the target sequences using real-time Polymerase Chain Reaction (PCR).

The XDive is intended for general laboratory use and must be used **exclusively by laboratory professionals trained in laboratory techniques and having studied the instructions for use of this instrument**.

No modification on this product is allowed.

Any modification may affect product performance and cause safety concern. The limited warranty is voided if any issue is resulted from any usage which is not specified by the intended use above, accident, abuse, misapplication, operation or storage outside of conditions as specified at section 2, or service or modification by someone other than Star Array personnel.

4. Preamble

Before setting-up operation of the XDive, it is important to read this User Manual thoroughly and completely. Non-observance of the instructions contained in this manual may entail safety hazards.

5. Symbols

Symbol	Heading	Description
	Warning	Noncompliance with instructions or procedures may lead to physical injury or even death or could cause damage to the instrument.
	Hot Surface	Potentially hot instrument surfaces.
	Biohazard	Certain precautions must be taken when working with potentially infectious material.
	Risk of Hand Crush	Risk of hand getting crushed by moving part(s).
	High Voltage	Risk of electric shock.
	Important Note	Important annotation.
	WEEE	Electrical and electronic equipment marked with this symbol are covered by the European directive WEEE. The symbol denotes that the equipment must not be disposed following the municipal waste system.

6. Safety Note, Warning and Precautions

The XDive must only be used by **trained and skillful personnel**. It is essential that the following safety information required for installation and operation of the XDive are carefully read and observed. Please assure that this safety information is accessible for every employee working with the XDive.

Warnings:

1. For In Vitro Diagnostic Use.
2. For Use Under Emergency Use Authorization (EUA) Only.
3. For Prescription Use Only.
4. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
5. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
6. This emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection

and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

	The XDive is an electromechanical instrument. There is a potential danger for the user of an electric shock or physical injury if the instrument is not used according to the instructions given in this manual. ➢ Follow all safety instructions printed on or attached to the instrument. ➢ Observe all general safety precautions which apply to electrical instruments. ➢ Do not access any electrical parts while the instrument is connected to the mains. ➢ Never touch switches or power cord with wet hands. ➢ Do not open the housing (the right-side panel is an exception) while the instrument is connected to the main power supply. ➢ Do not open the thermal chamber during operation. ➢ Do not pour fluids into the thermal chamber. ➢ Never clean the instrument without turning the instrument power switch off and disconnecting the power cord. ➢ Do not manipulate the instrument. ➢ Only authorized service personnel are allowed to perform service or repairs required for this unit. ➢ Danger of explosion through sparks. Keep all potentially inflammable or explosive material (for example, anesthetic gas) away from the instrument. Spraying liquid on electrical parts can cause a short circuit and result in a fire. Keep the cover closed while the instrument is connected to the mains and do not use sprays in the vicinity of the XDive. During firefighting operations, disconnect the XDive from the mains ➢ For protection against electrical shock hazards, the instrument must be directly connected to an approved power source such as a three-wire grounded receptacle for the 230V line. Where an ungrounded receptacle is encountered, a qualified electrician must replace it with a properly (PE) grounded receptacle in accordance with the local electrical code. An extension must not be used. Any break in the electrical ground path, whether inside or outside the instrument, could create a hazardous condition. Under no circumstances should the user attempt to modify or deliberately defeat the safety features of this instrument. If the power cord becomes cracked, frayed, broken, or otherwise damaged, it must be replaced immediately with the equivalent part from Star Array.
	➢ The instrument may not be used to analyze infectious materials unless additional safety measures to ensure safe sample handling (e.g., placing the instrument in a biological safety cabinet) are taken beforehand. ➢ Although working with highly purified nucleic acids, please regard for your own safety all biological material as potentially infectious. Handling and disposal of such material should be performed according to local safety guidelines. Spills should be immediately disinfected with an appropriate disinfectant solution to avoid spreading contamination to laboratory personnel or equipment. ➢ Always wear safety goggles and gloves when dealing with toxic, caustic or infectious materials.
	➢ (When the right-side panel is removed) The thermal tanks and chamber are hot while the instrument is operating.

	(Location: Inside of front door)  ➢ The gaps beside door hinges might trap finger. User is advised to ensure no object is nearby while accessing the front door.
	(Location: Rear panel)  ➢ Before turning on the power, ensure that the power cord is properly connected. ➢ When the power is on, ensure that the power cord is properly connected at all time. ➢ Do not let any conductive fluid contact this area.

7. System Description

Working Principle of the XDive Machine

The XDive Machine is a rapid thermal cycler with integrated real-time detection capabilities. This set-up enables homogeneous PCR to be performed, i.e., simultaneous amplification and detection of target nucleic acids. Detection of target nucleic acid is performed by adding either a fluorescent double-stranded-DNA-specific dye or sequence-specific oligonucleotide probes labeled with fluorophores. Both approaches allow measuring the generation of PCR products during amplification, the basis of quantitative PCR (qPCR). The possibility to freely combine four excitation and four emission filters allows analysis of signals from multiple dyes in multiplex PCR assays.

8. Control Software Information

Software Title: XDive
Software functions:

1) Set operating parameters.
2) Control & operate XDive Machine.
3) Capture & analyze test data.

Detail instructions are in section 9.

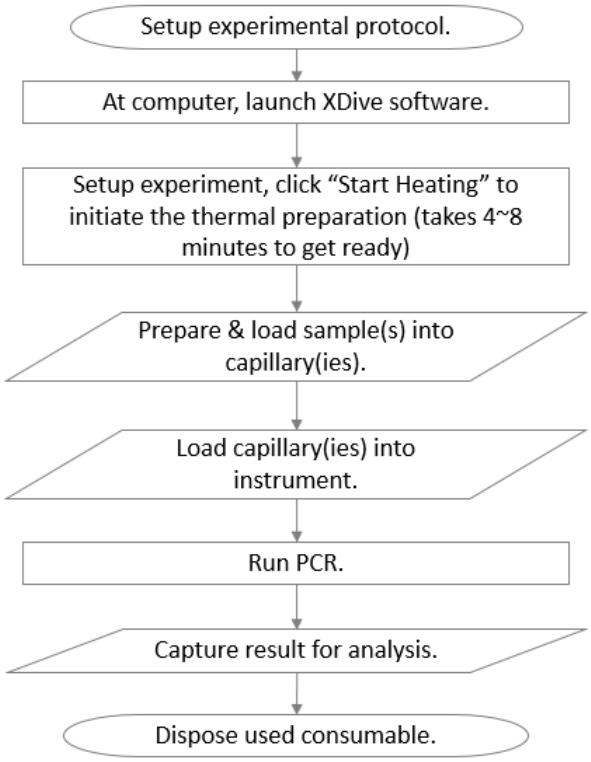
<u>Minimum System Requirements</u>

- Operating System: Microsoft Windows 10
- Processor Speed: 2.2 GHz
- Memory, a.k.a. RAM: 8 GB
- Hard Disk Space: 128 GB
- Monitor Size: 15” (best 21”)

Date of latest software update: 31-Mar-22

9. Operating Procedure

9.01 Operating Process Flow



9.02 Check / Inspection Before Use

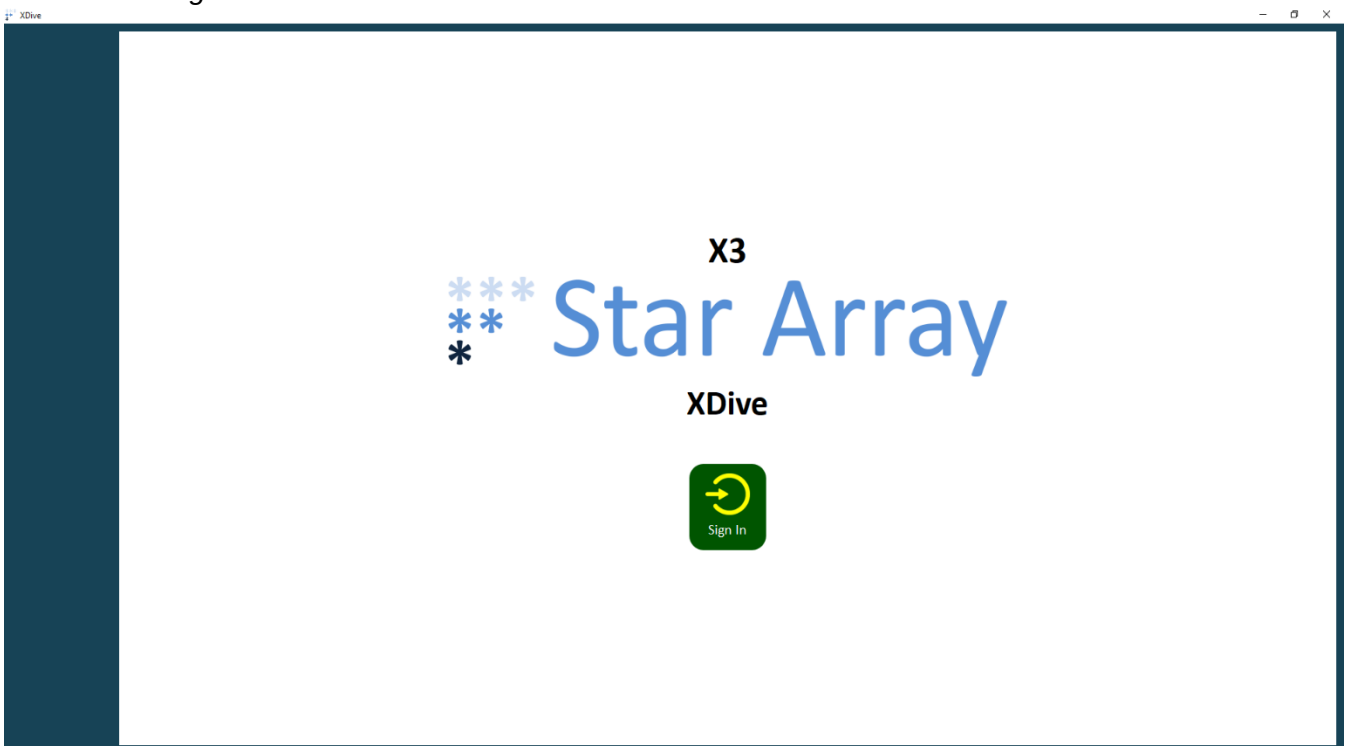
9.02.01 Ensure all connectors between XDive Machine and computer are properly connected.

9.03 System Start-Up

9.03.01 Ensure XDive Machine has been physically connected with computer & power source, then switch on XDive Machine.

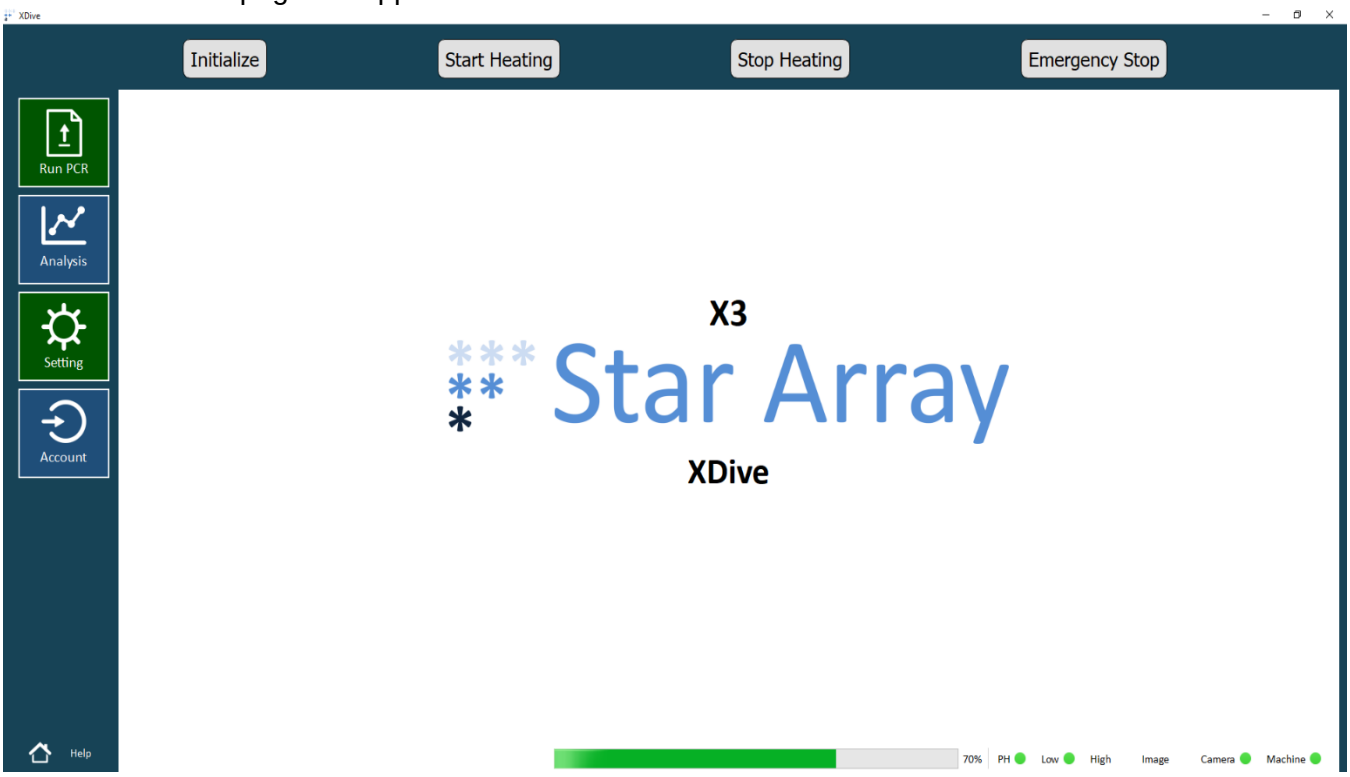
9.03.02 At "Start" button, desktop or taskbar, click to launch the "*XDive*" software.

9.03.04 Click "*Sign In*".



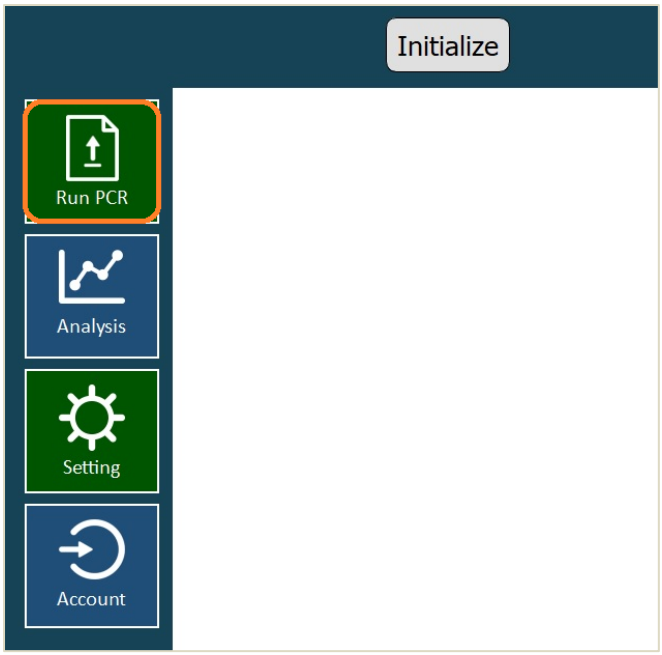
Key in password when necessary.
The default User Name & Password are both "*admin*".

9.03.05 The main page will appear.



9.04 To load & run script

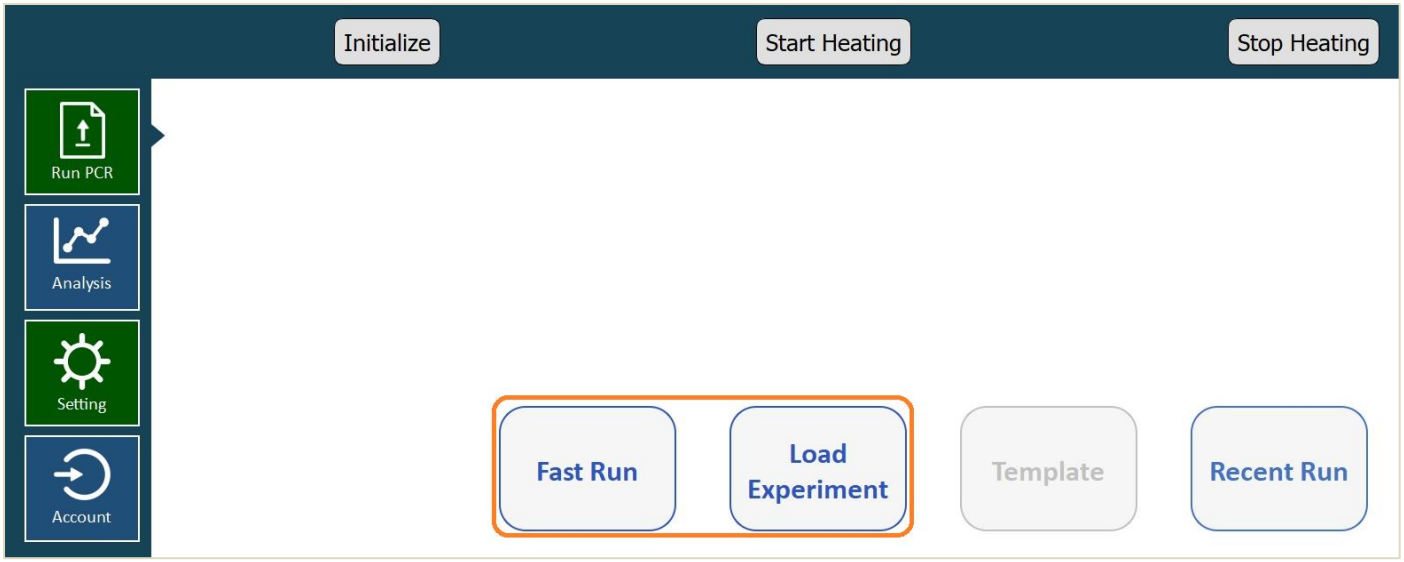
9.04.01) Click "*Run PCR*" on the left panel.



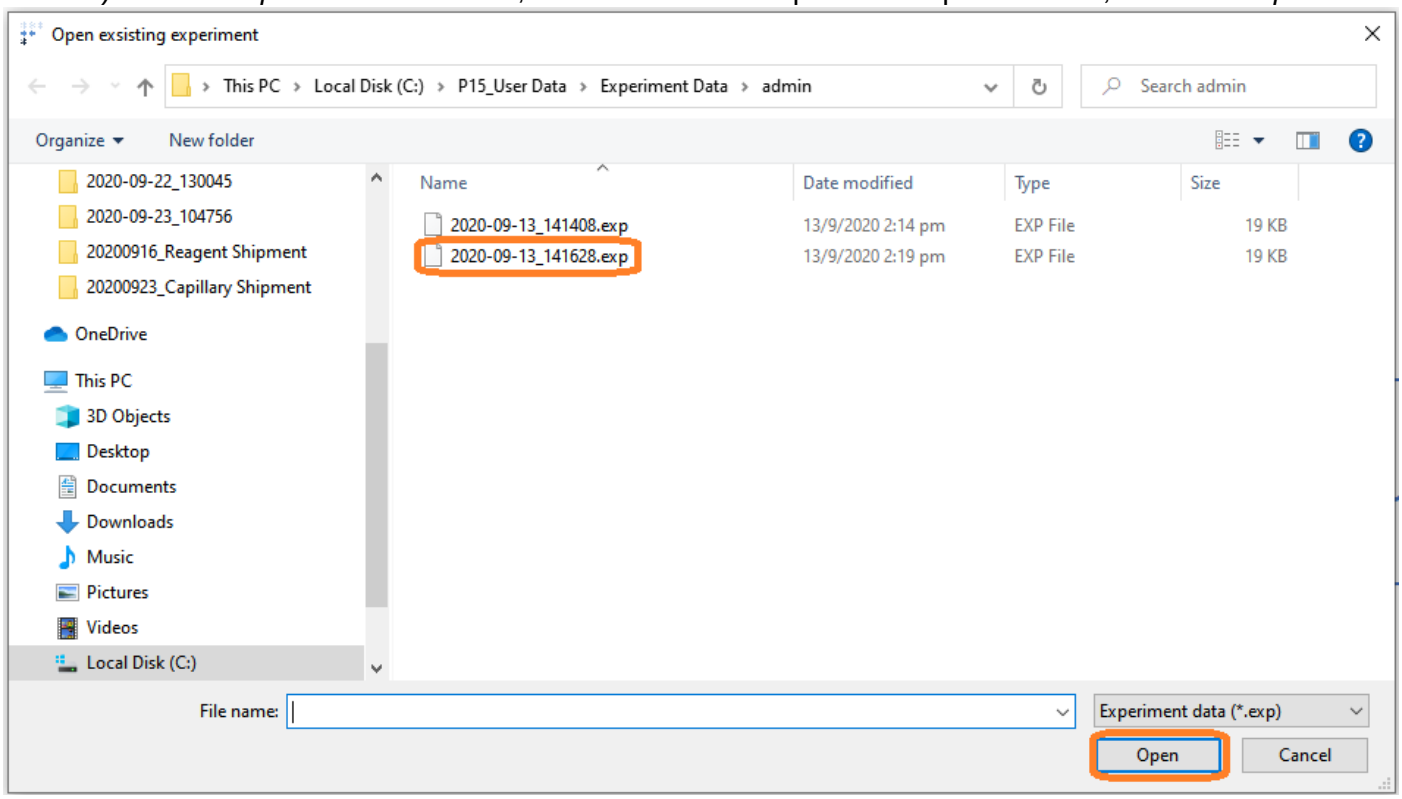
9.04.02) Click "*Fast Run*".

User can also re-run previous protocol by clicking "*Load Experiment*".

The "*Recent Run*" button brings user back to the ongoing experiment setup.

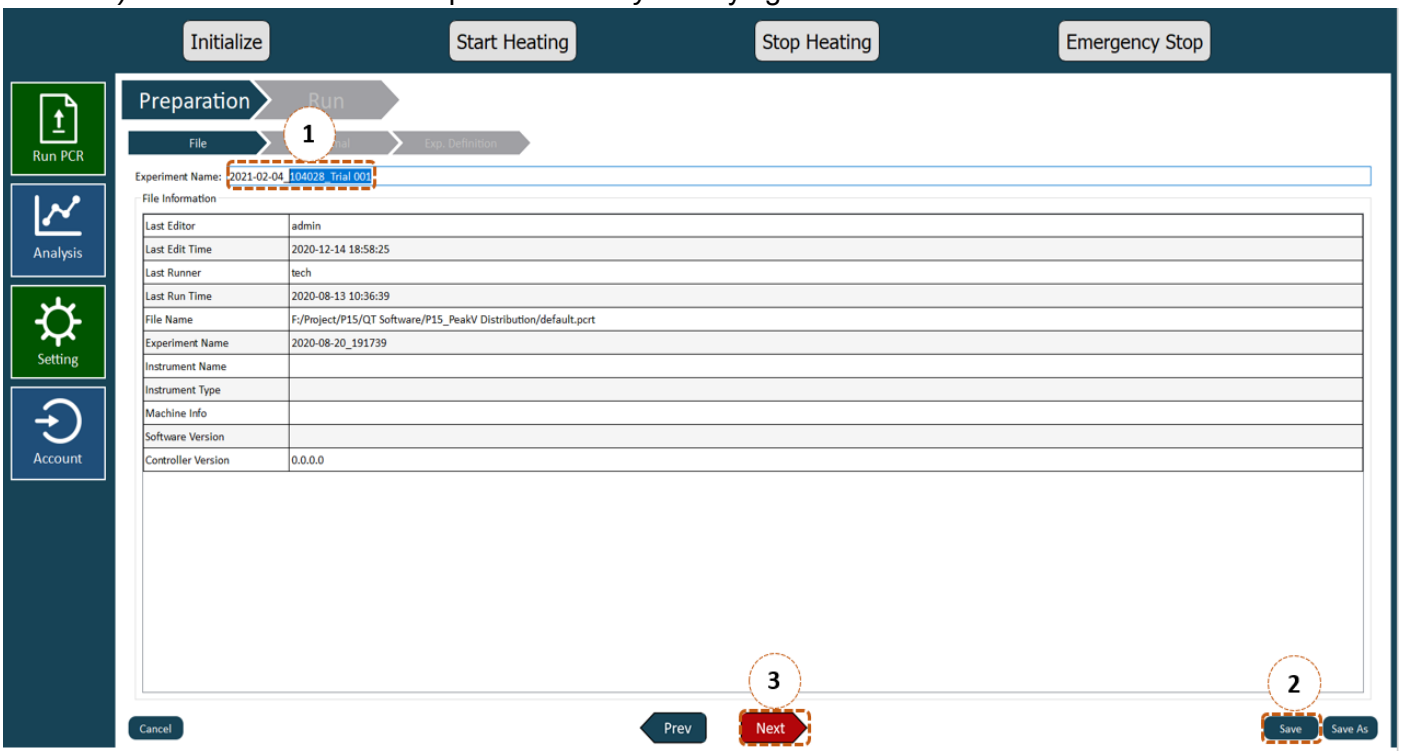


9.04.03) If "*Load Experiment*" is chosen, then browse for the previous experiment file, and click "*Open*".

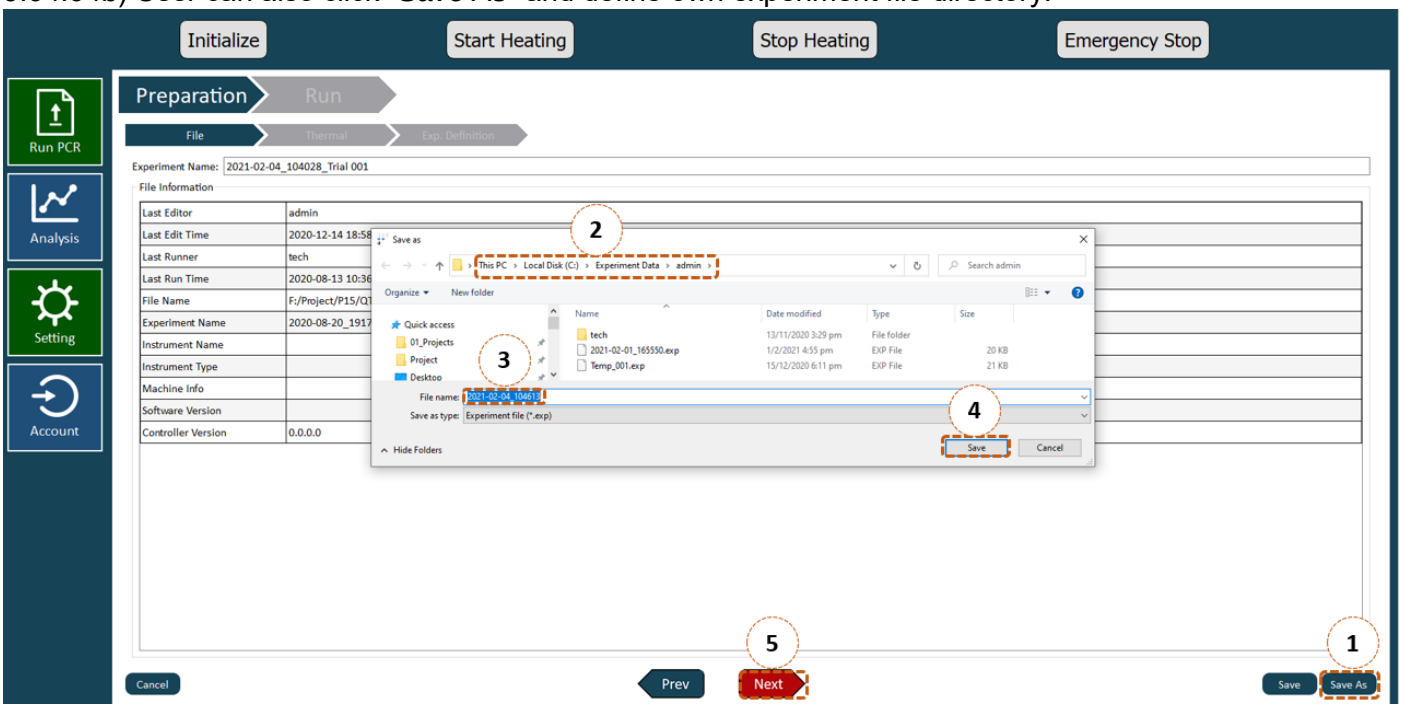


Remark: Both past experiment setting and test data are in "*.exp*" format.
Tip: By observing the file size, user can estimate the length of the run.

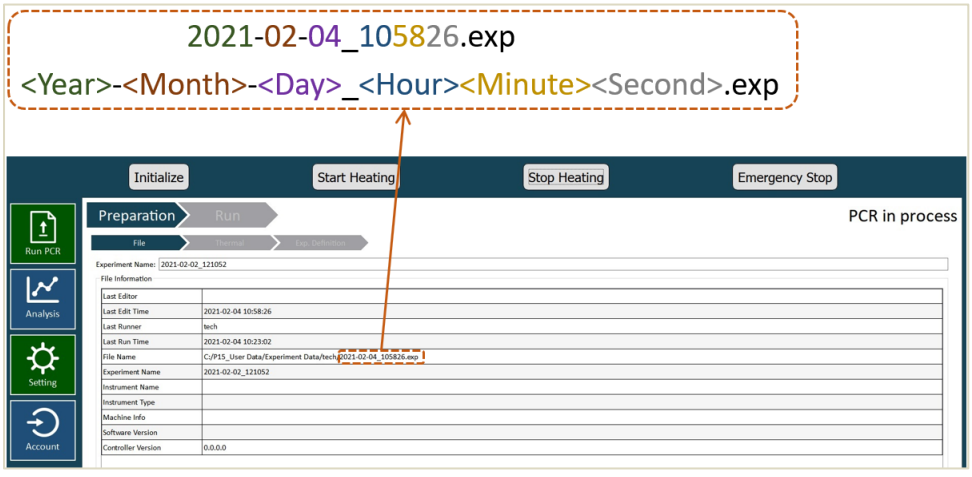
9.04.04a) User can rename the experiment file by modifying the "*File Name*" then click "*Save*" and "*Next*".



9.04.04b) User can also click "*Save As*" and define own experiment file directory.



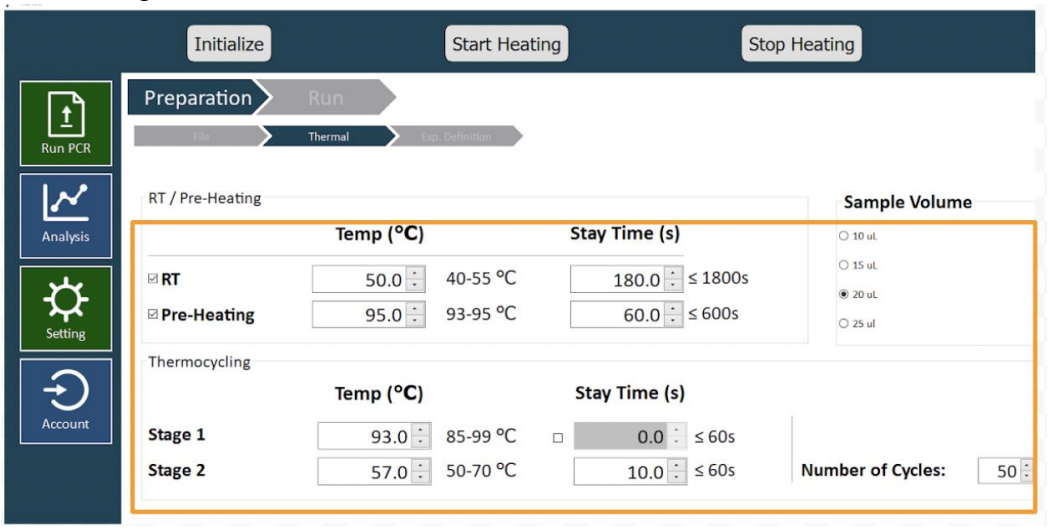
9.04.04d) The default file name of the experiment data is automatically generated at the "*Experiment Name*" field when the PCR Run is started.



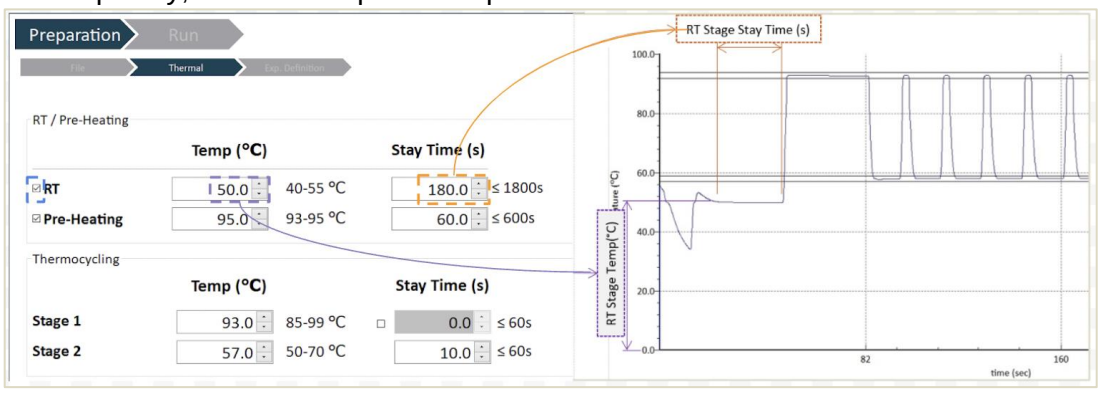
The experiment file is also automatically generated at the directory stated in the "*File Name*" field.

9.04.05) Make the selection according to user's requirement / IFU.

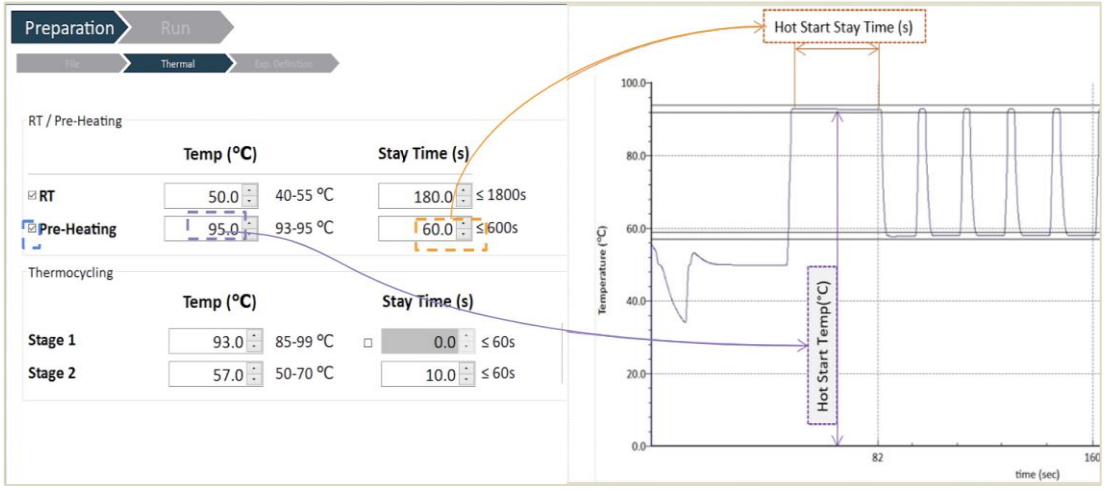
Note: In this current software version, no additional stage can be inserted on top of these 4 existing stages.



9.04.05a) Click the check box beside "*RT Stage*" if Reverse Transcriptase is required. Subsequently, enter the required temperature and duration.

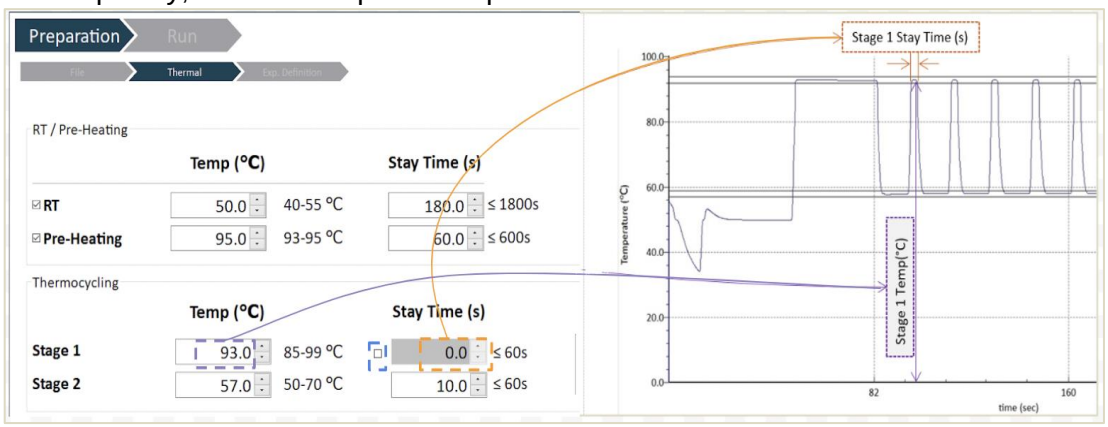


9.04.05b) Click the check box beside "*Pre-Heating*" when required. Subsequently, enter the required temperature and duration.

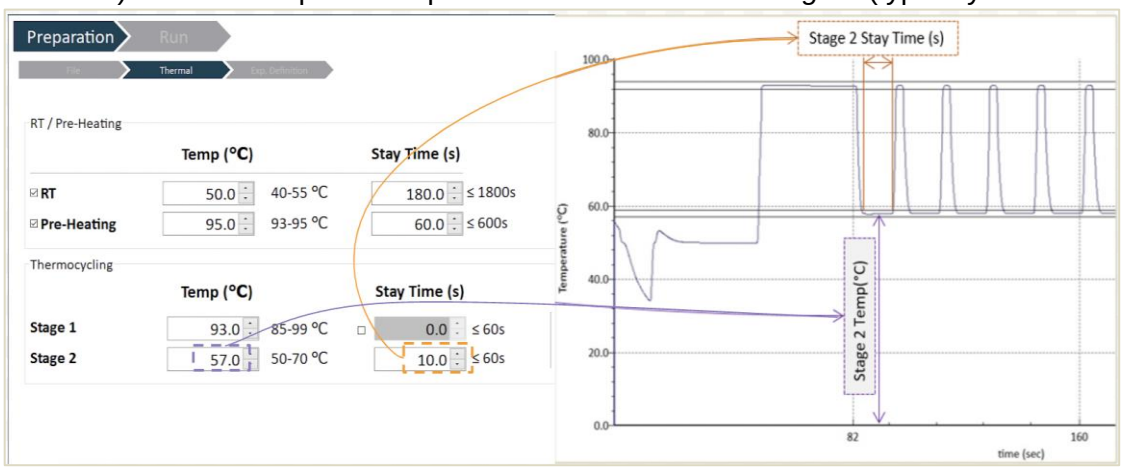


9.04.05c) Click the check box at the same row as "*Stage 1"* (typically for Denaturation) when Stay Time is required.

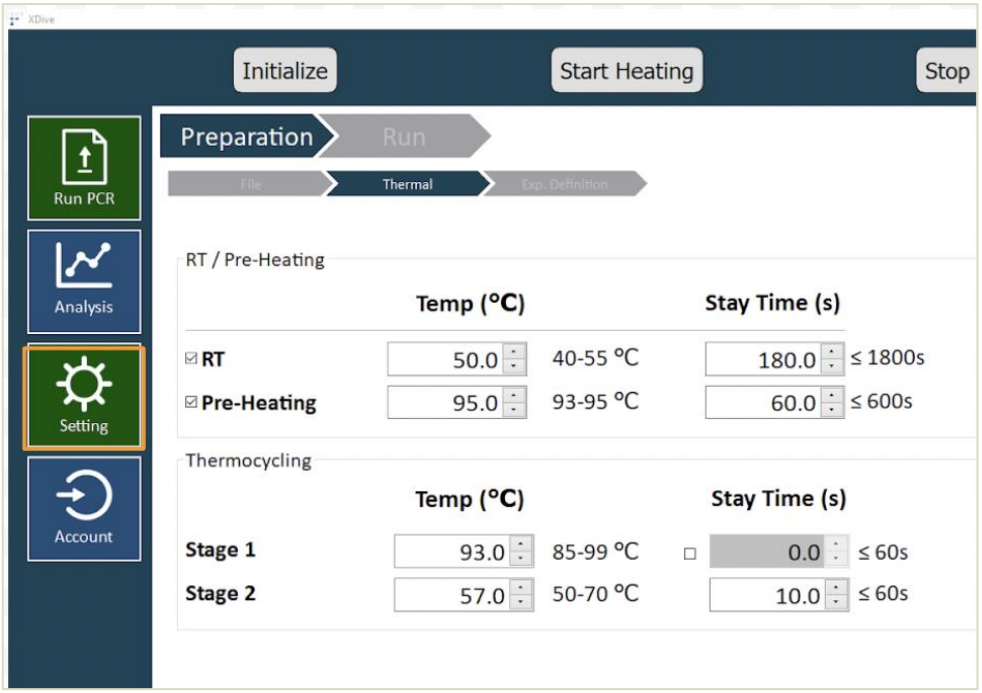
Subsequently, enter the required temperature and duration.



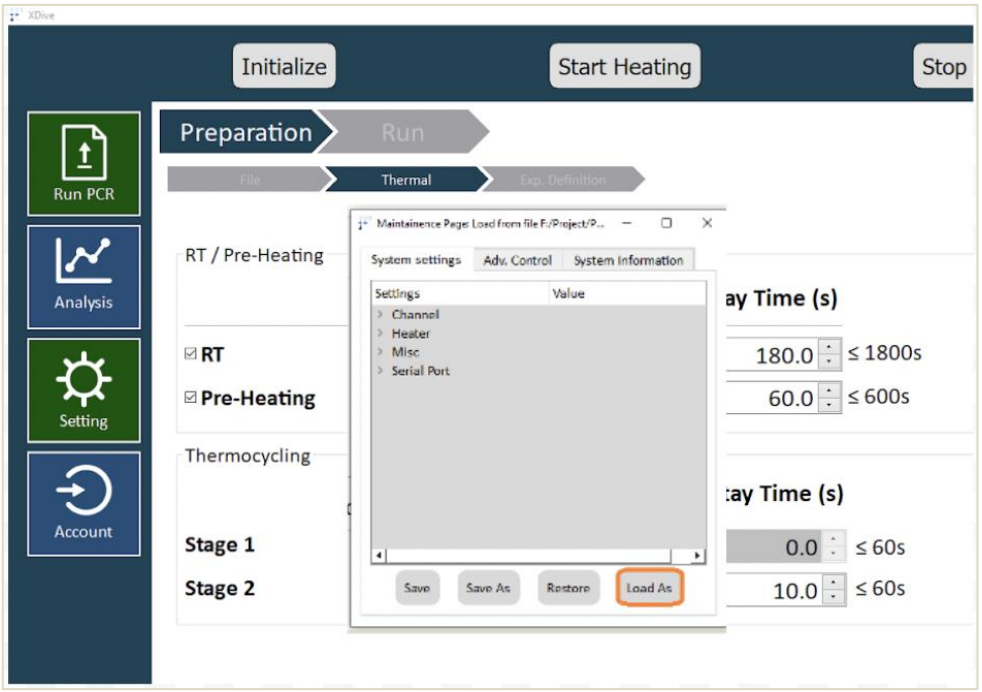
9.04.05d) Enter the required temperature and duration for *Stage 2* (typically for Annealing and Extension).



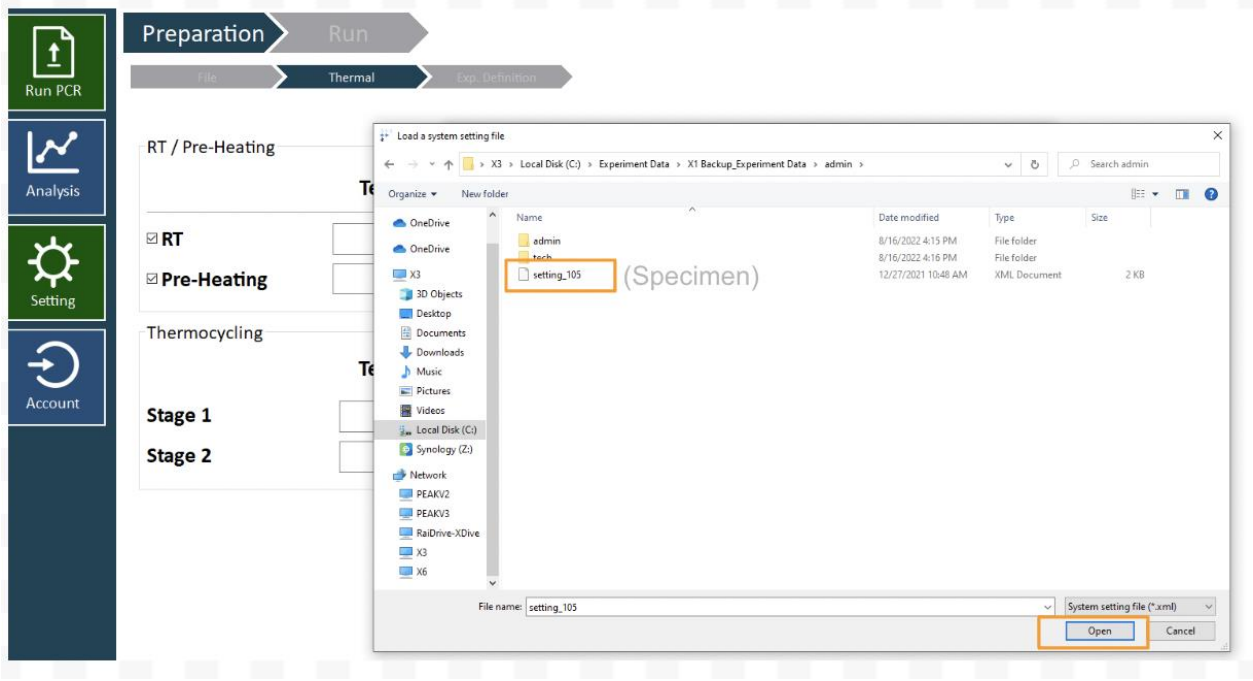
9.04.06a) If a different set of setting parameters is needed for the reaction condition of this PCR run, and the manufacturer has already created a set of preset parameters for user, then click "*Setting*".



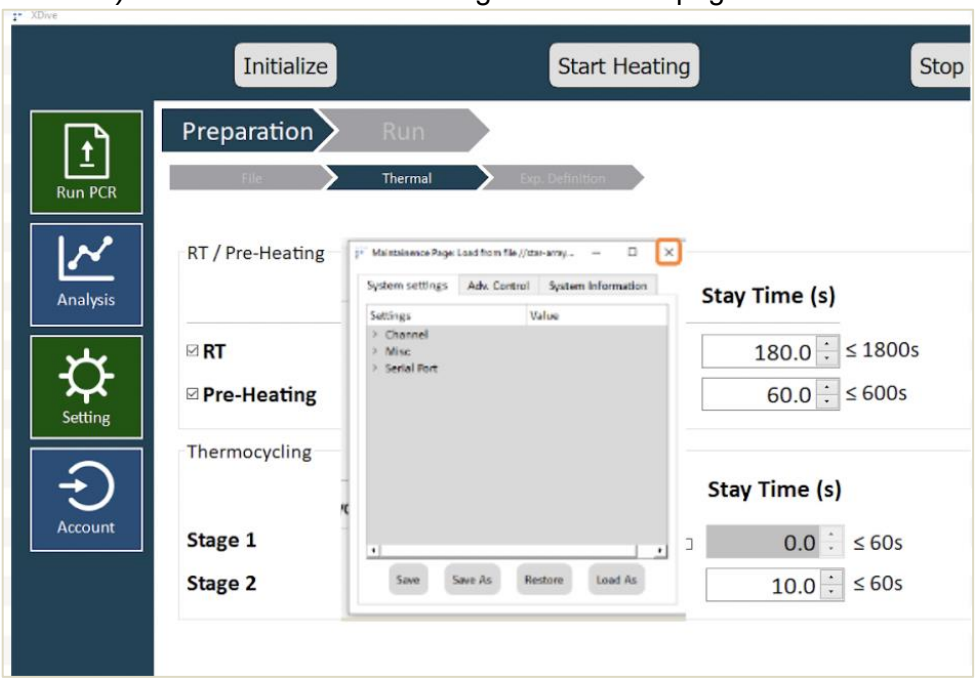
9.04.06b) Click "*Load As*".



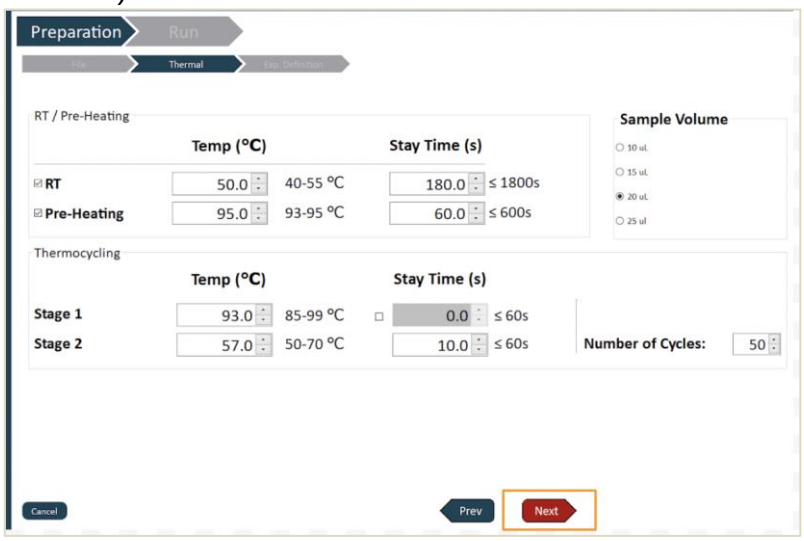
9.04.06c) Browse for the preset parameter created by the manufacturer, then click "*Open*".
The preset parameter can be created by the manufacturer upon user's request to accommodate certain unusual combination of reaction volume, or temperature profile, or both.



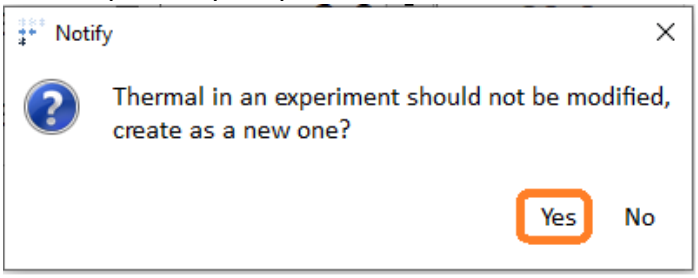
9.04.06d) Click "x" to close the setting maintenance page window.



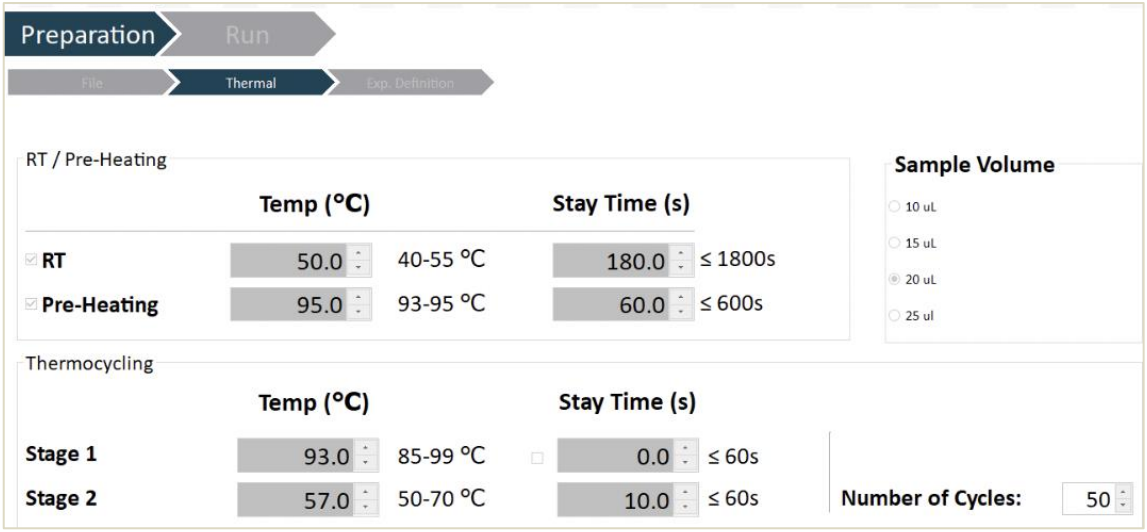
9.04.06e) Click "*Next*".



9.04.06f) When prompted whether to create a new experiment, user can click "*Yes*".



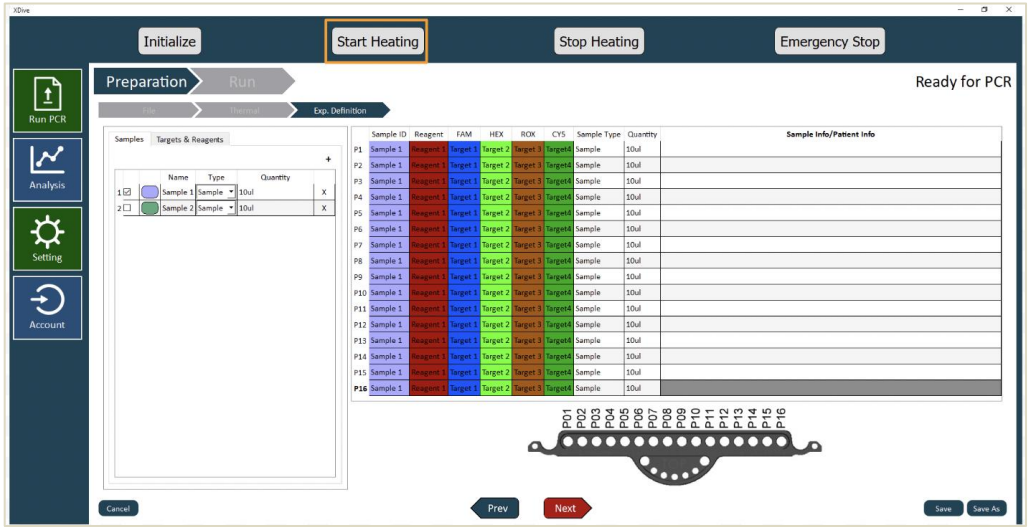
9.04.06g) After user clicks "▶" (Run) mentioned at step 9.04.13, all the thermocycling parameters will be locked and cannot be modified.



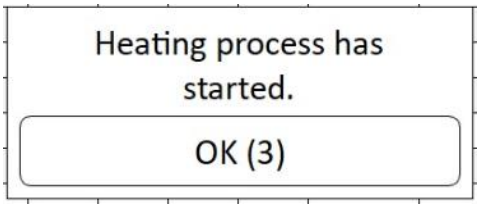
Hence, user is suggested to confirm each parameter before clicking "▶".

To load / unload capillary that contains sample, please refer to section 9.05 "*To Load / Unload Capillary That Contains Sample*" in this document.

9.04.07a) Click "*Start Heating*".

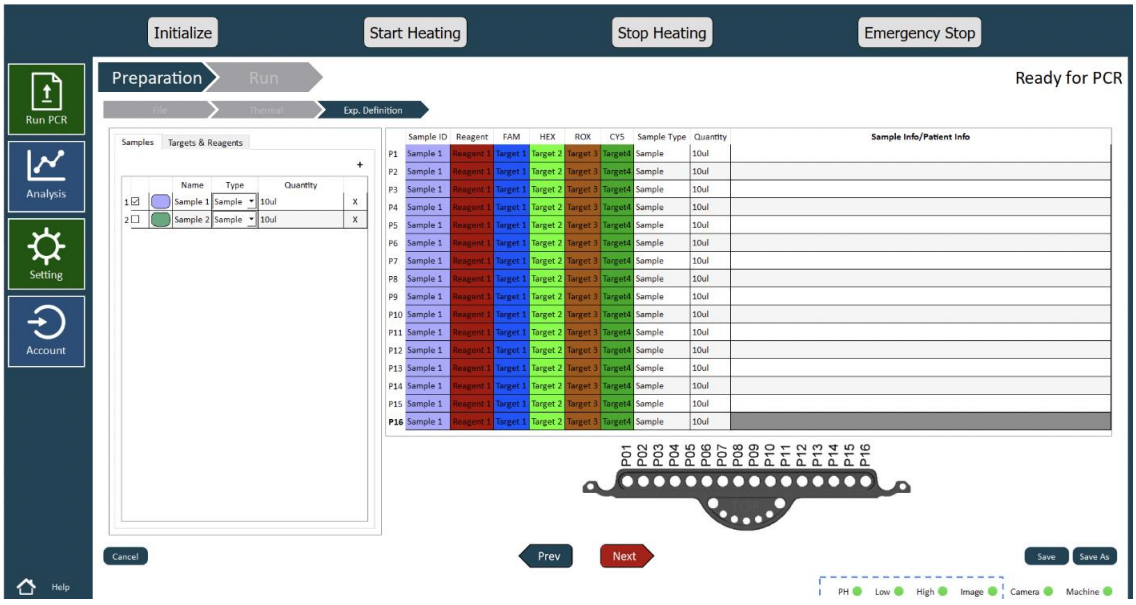


9.04.07b) When a message box pops up, user can either click "*Ok*" or wait for the message to automatically disappear after 3 seconds.

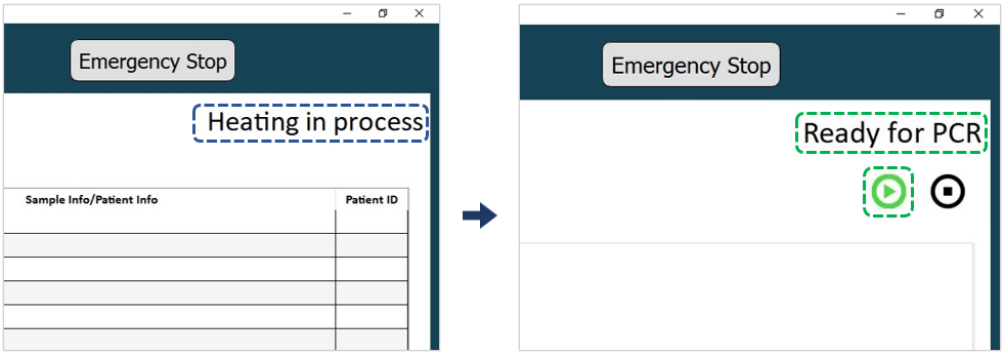


9.04.07c) The typical initial heat up time is 4~9 minutes.
The indicators of Heaters at the lower right corner shows the correspondent status:

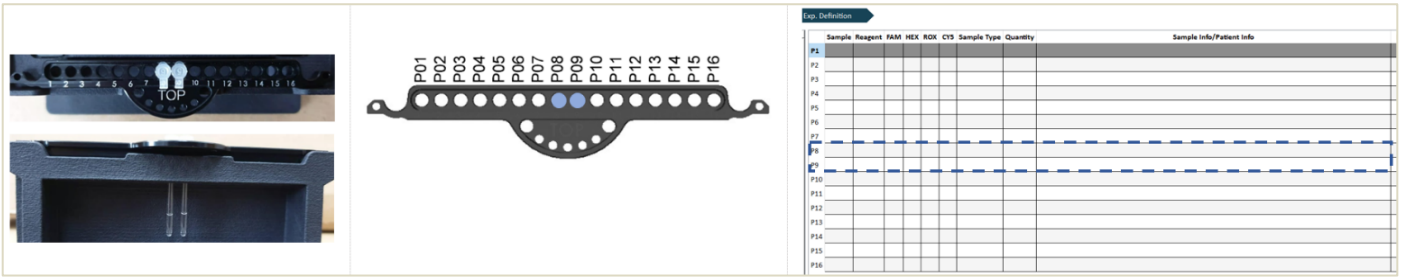
i) Green Blink : Tanks are being heated / cooled towards the target temperatures.
ii) Green Steady : Tank temperatures have reached the target ranges.
iii) Red Steady : Auto-PID in progress.
iv) Grey Steady : No heating / cooling.



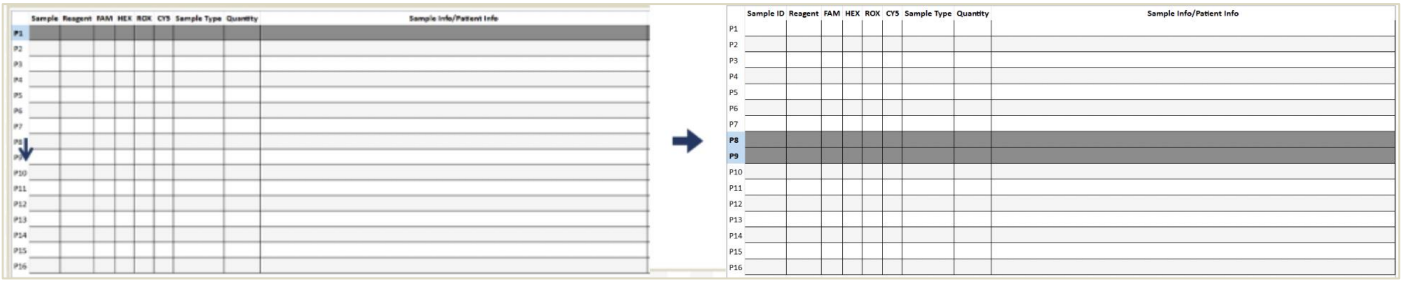
9.04.07d) When the status displayed on the upper right corner changes from "*Heating in process*" to "*Ready for PCR*", the instrument is ready for user to initiate PCR Run.



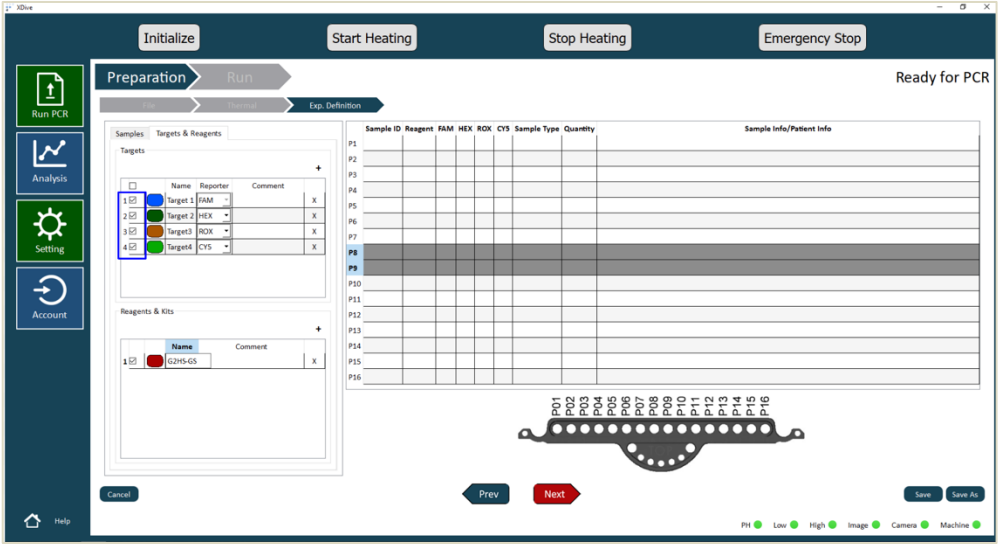
9.04.08a) At the "*Exp. Definition*" tab, select the position that user physically loads reagent onto.
In this case, for example, position P08 and P09.



9.04.08b) Click on "*P8*", then drag on to "*P9*". Both positions will be selected.
Alternatively, user can also perform multi-selection by holding either "*Shift*" or "*Ctrl*" key on the keyboard and left-clicking the desired position at the same time.



9.04.08c) Under the "*Targets*" section, tick the checkboxes correspondent to the target(s) required.
In this case, all four were selected.



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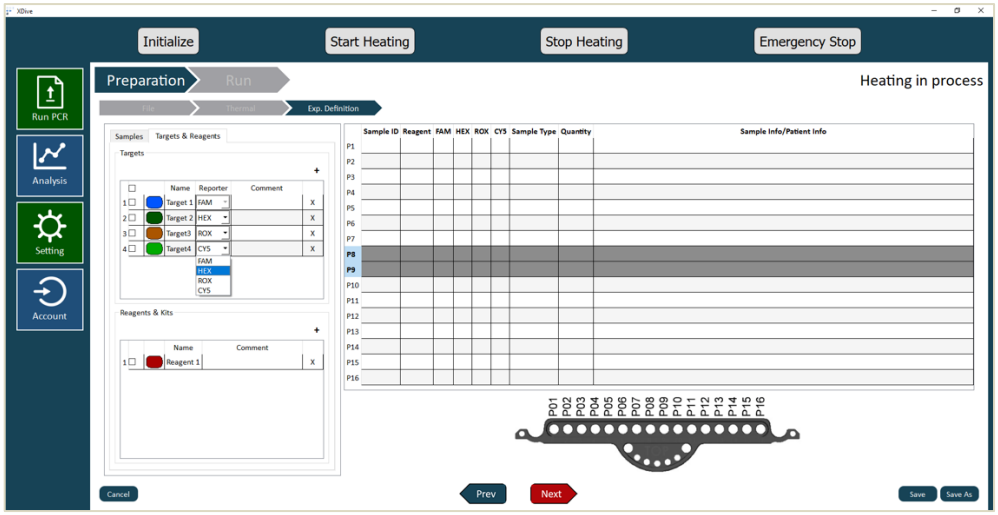
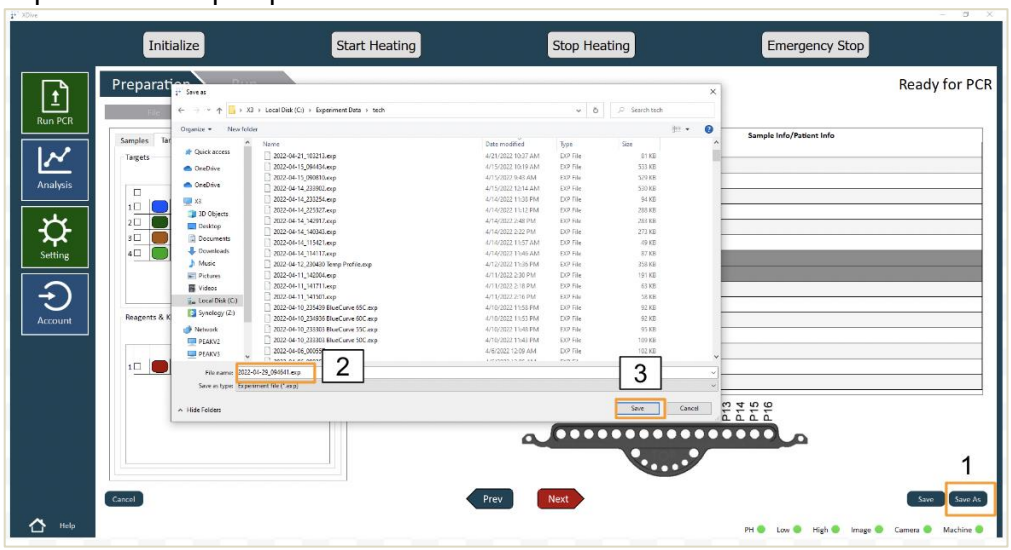
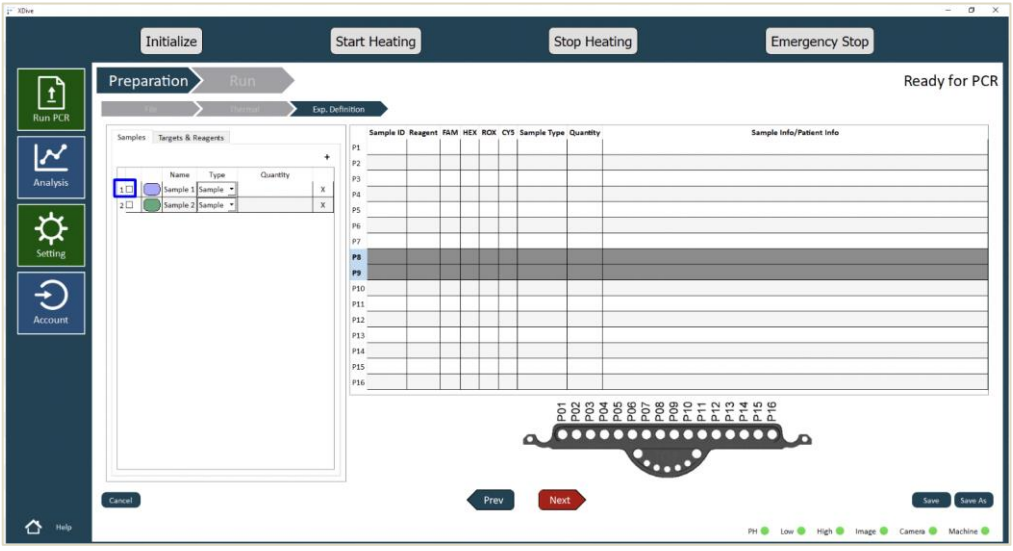
There are 4 Reporter options are available.

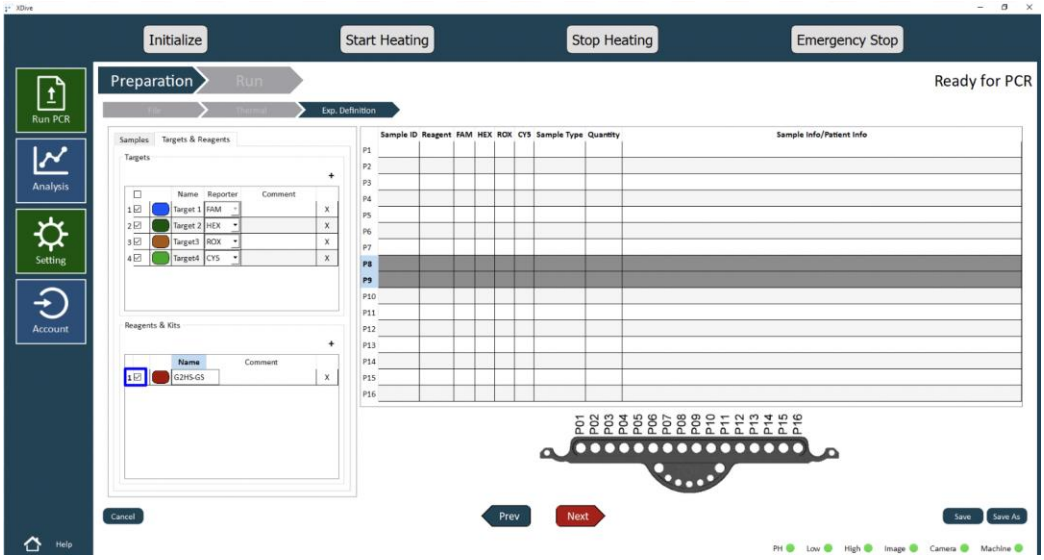


9.04.08d) If the left panel interface shows no response to any click, then click "*Save As*" to save this experiment setup to proceed.

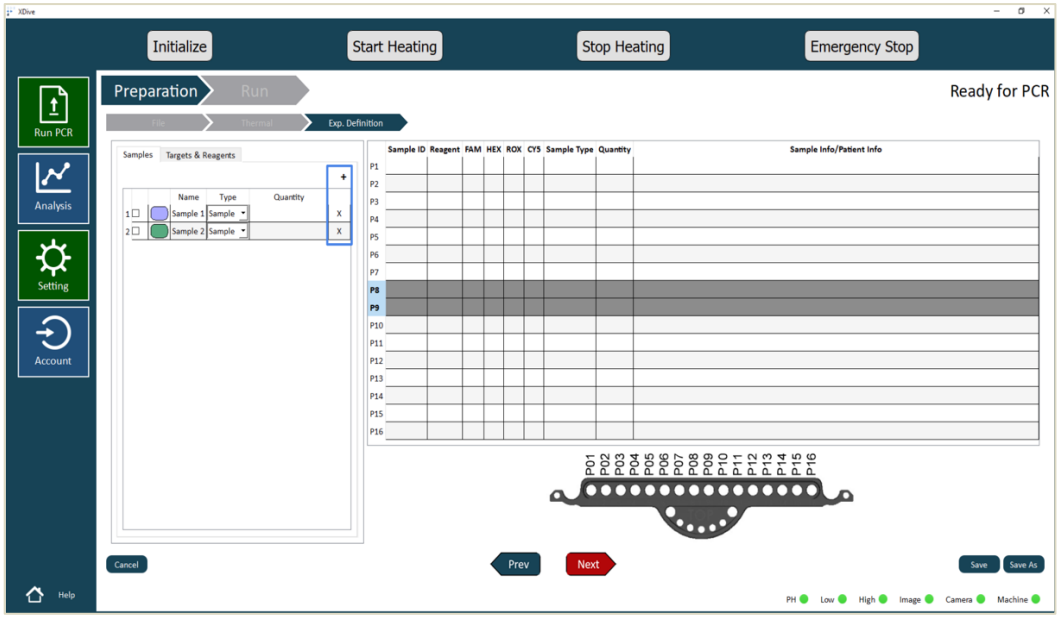


9.04.08e) Under "*Samples*" & "*Reagent*", click the required selections.



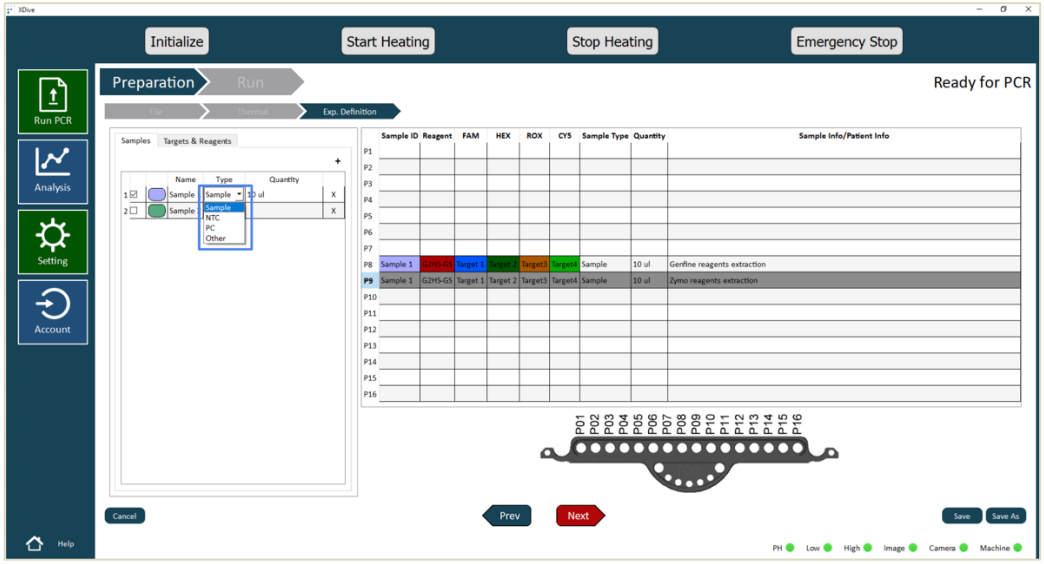


9.04.08f) Under the left 3 sections, user can add selection(s) by clicking “+” and remove selection(s) by clicking the “x” sign(s) beside the correspondent selection(s).

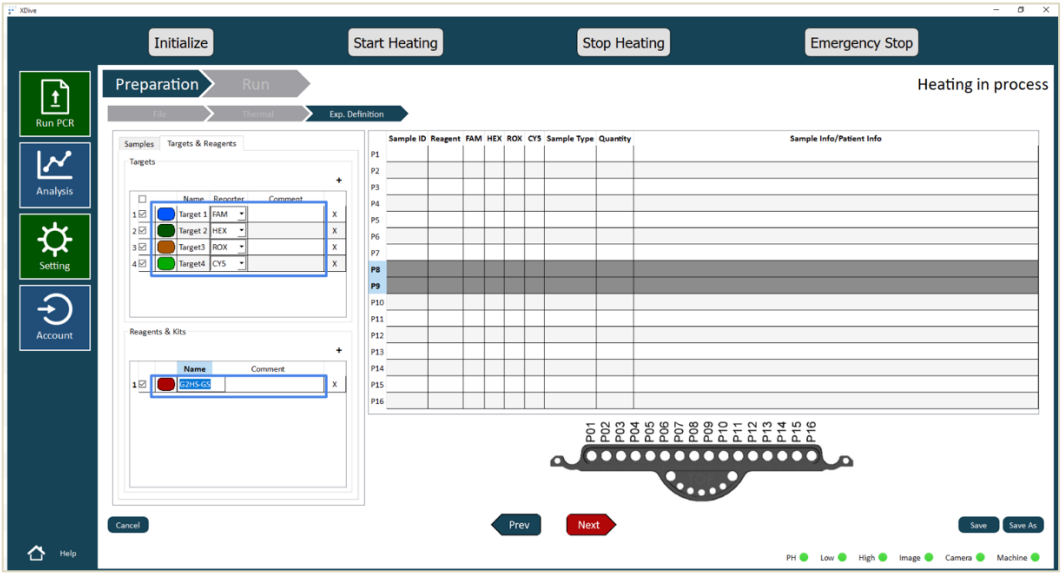


9.04.08g) Under the Sample Type,

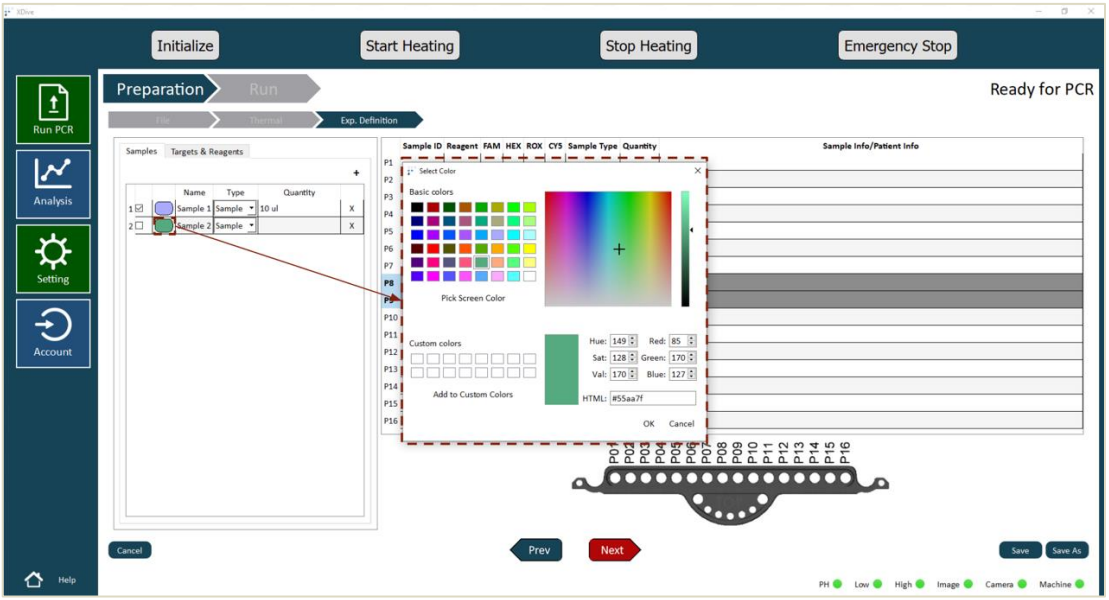
- *NTC* – No Template Control
- *PC* – Positive Control



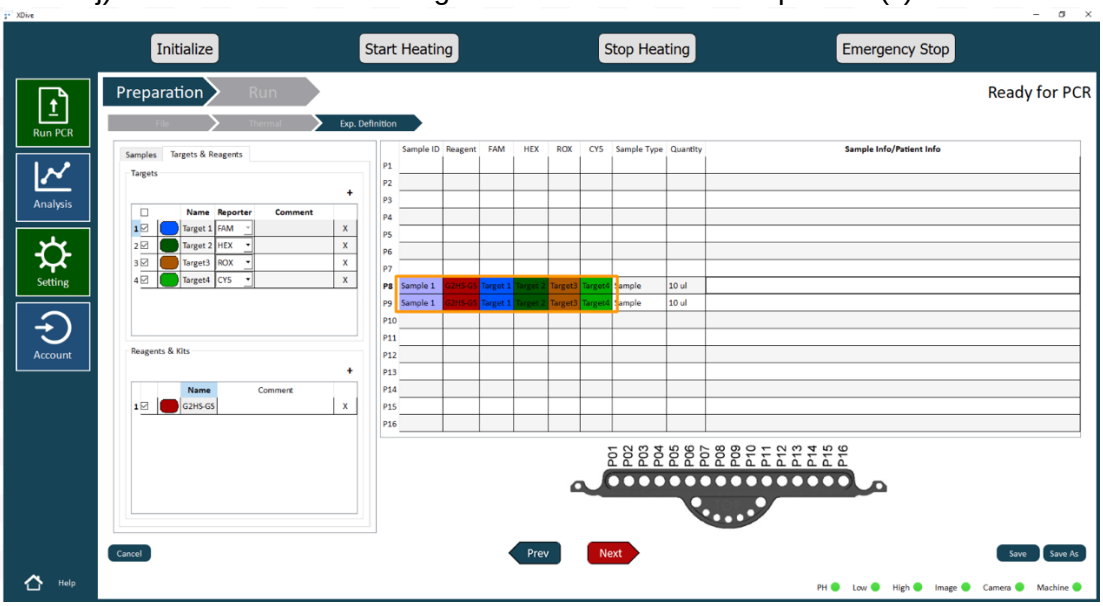
9.04.08h) User can also edit the Name, Quantity or Comment of each option by double-clicking them.



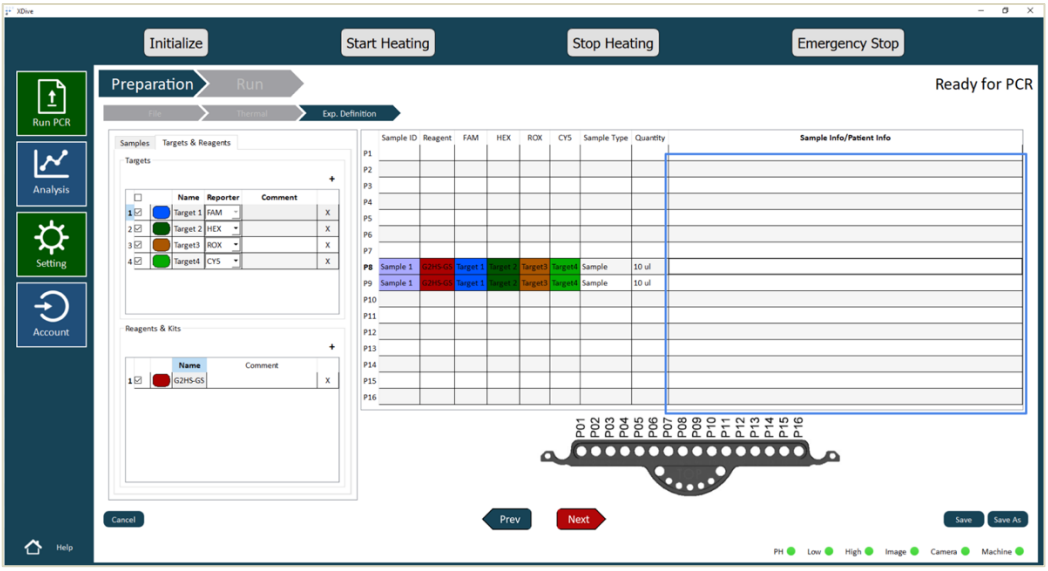
9.04.08i) By clicking on the color button, user can change the color of the Targets, Samples and Reagent & Kits.



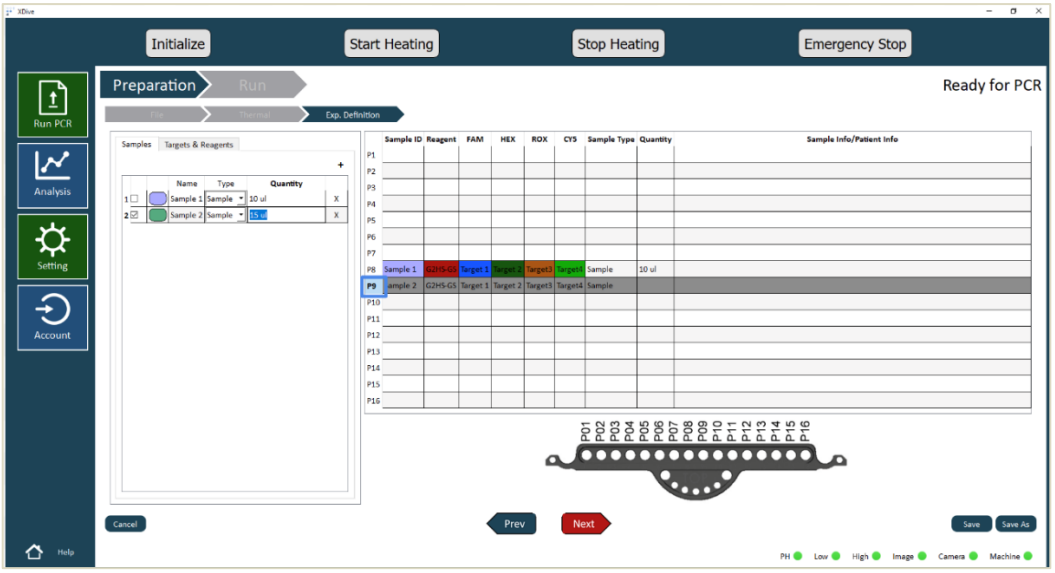
9.04.08j) The selections will be registered onto the selected position(s).



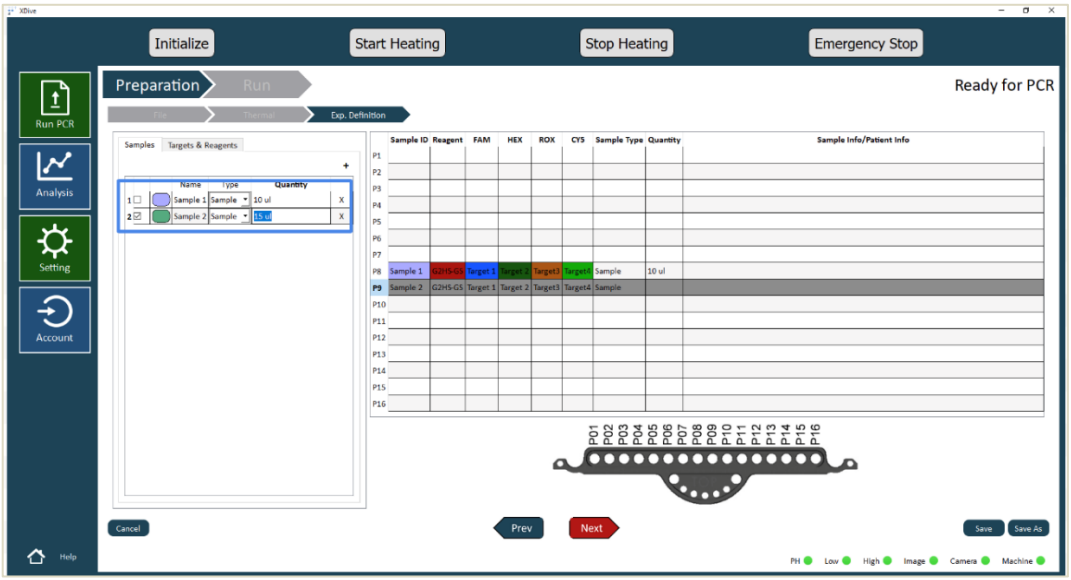
9.04.08k) The fields under "*Sample Info/Patient Inf o*" column can be added / edited after a double-click.



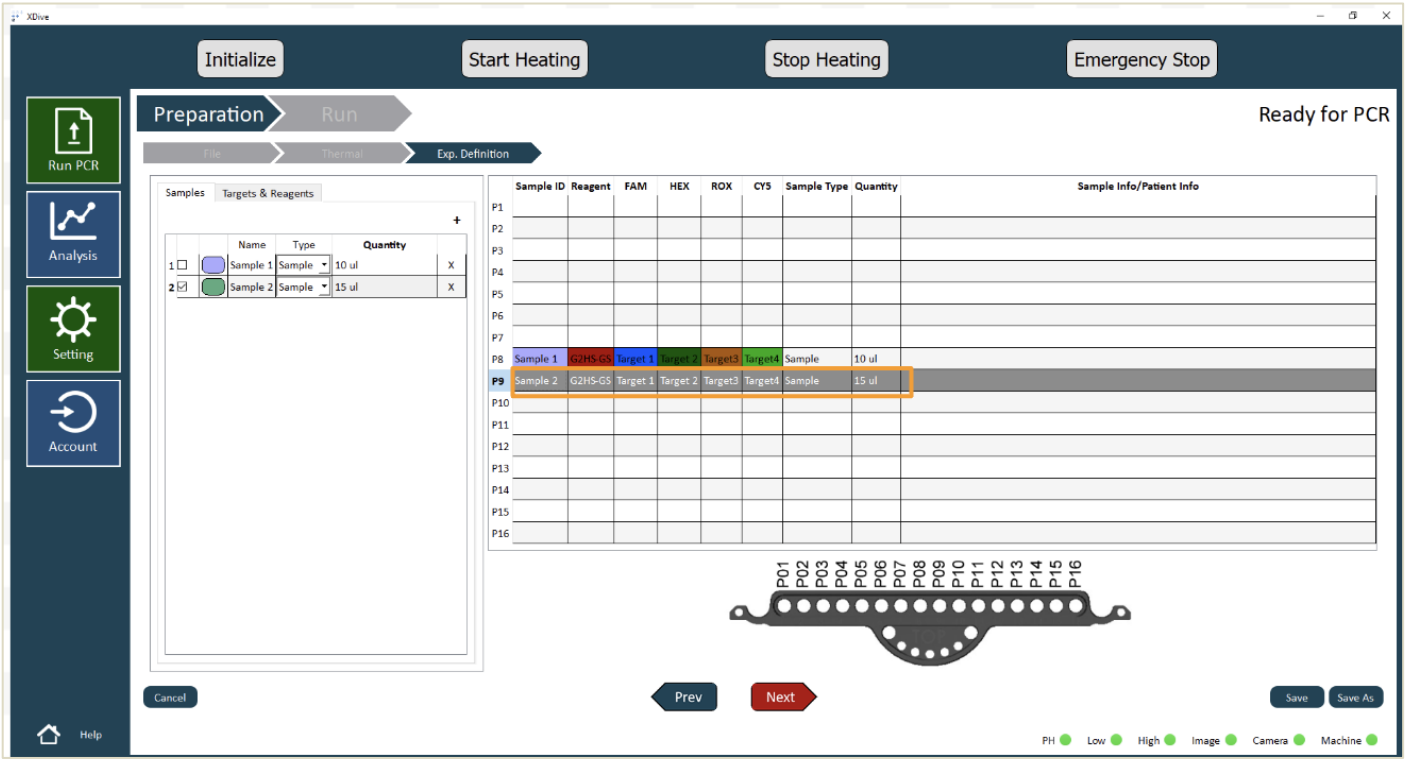
9.04.09a) To edit the existing position selection, click on the position number.
In this case, for example, position "*P9*".



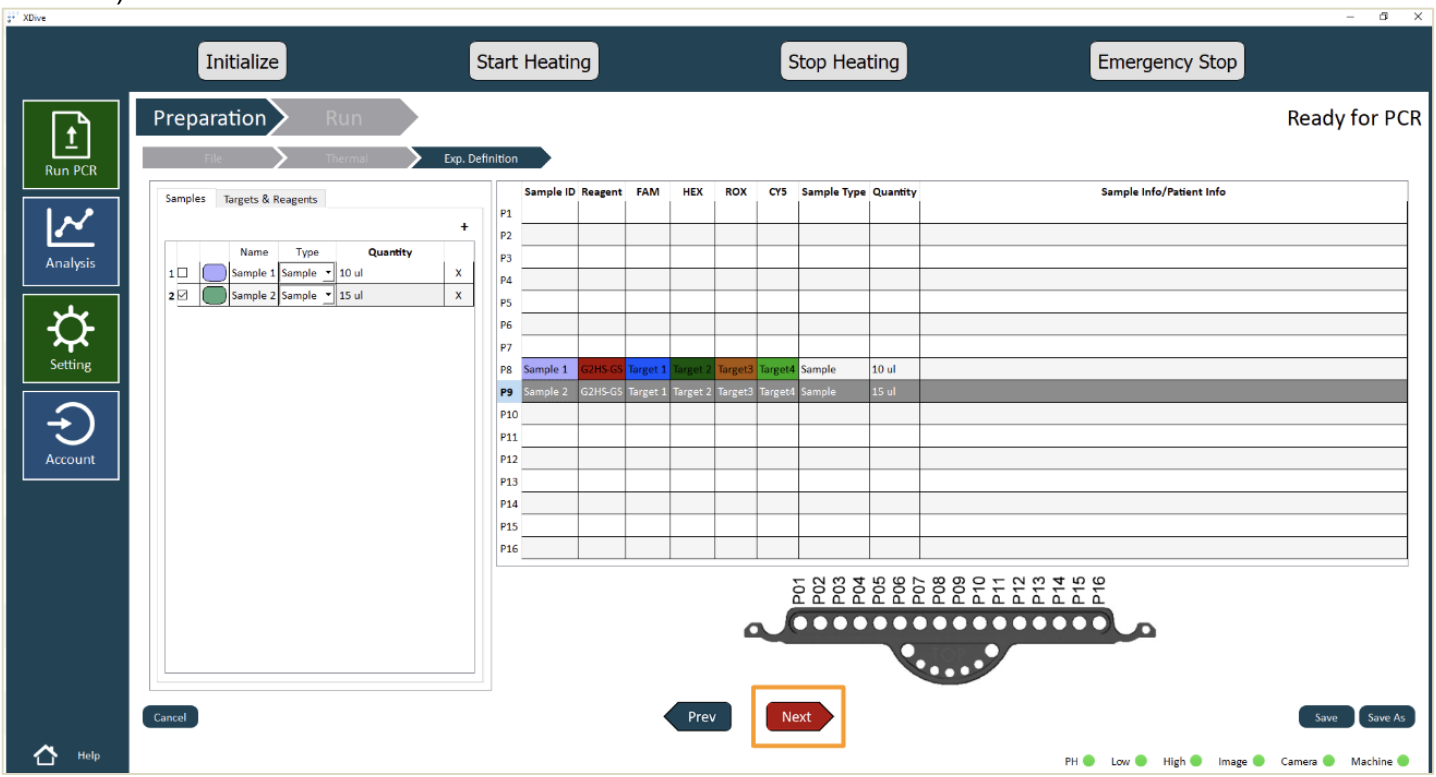
9.04.09b) Make the required change on the left panel.



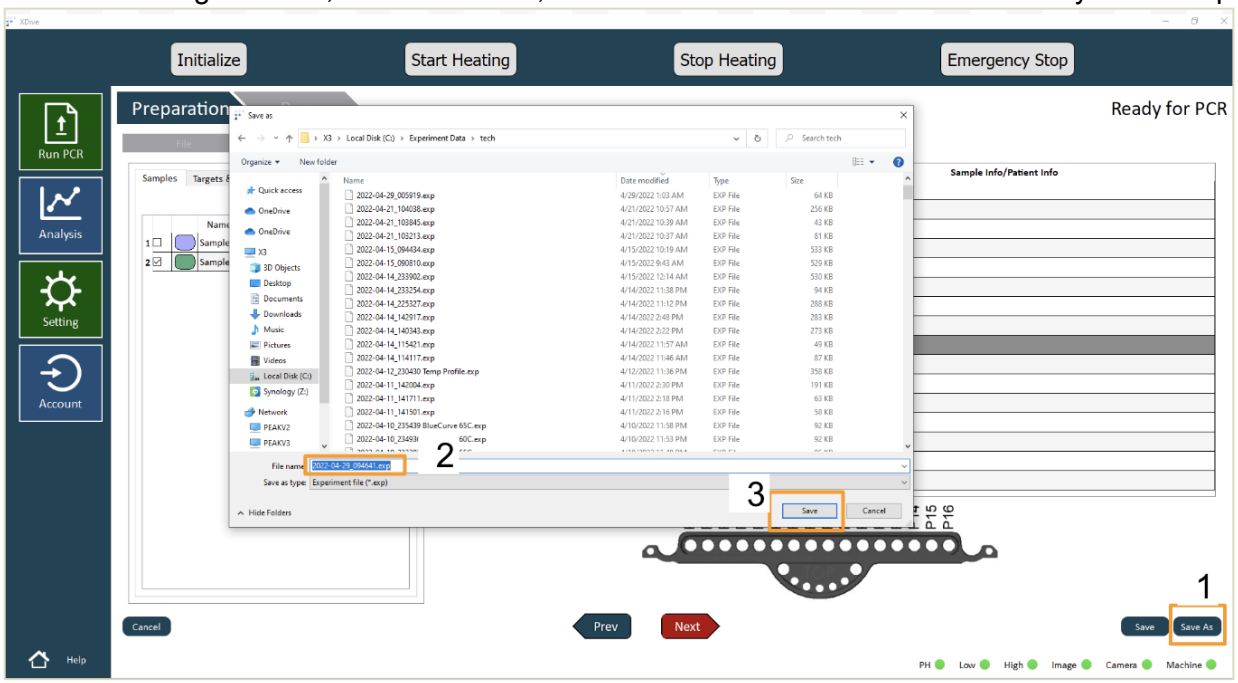
9.04.09c) The changes will be applied to the selected position.



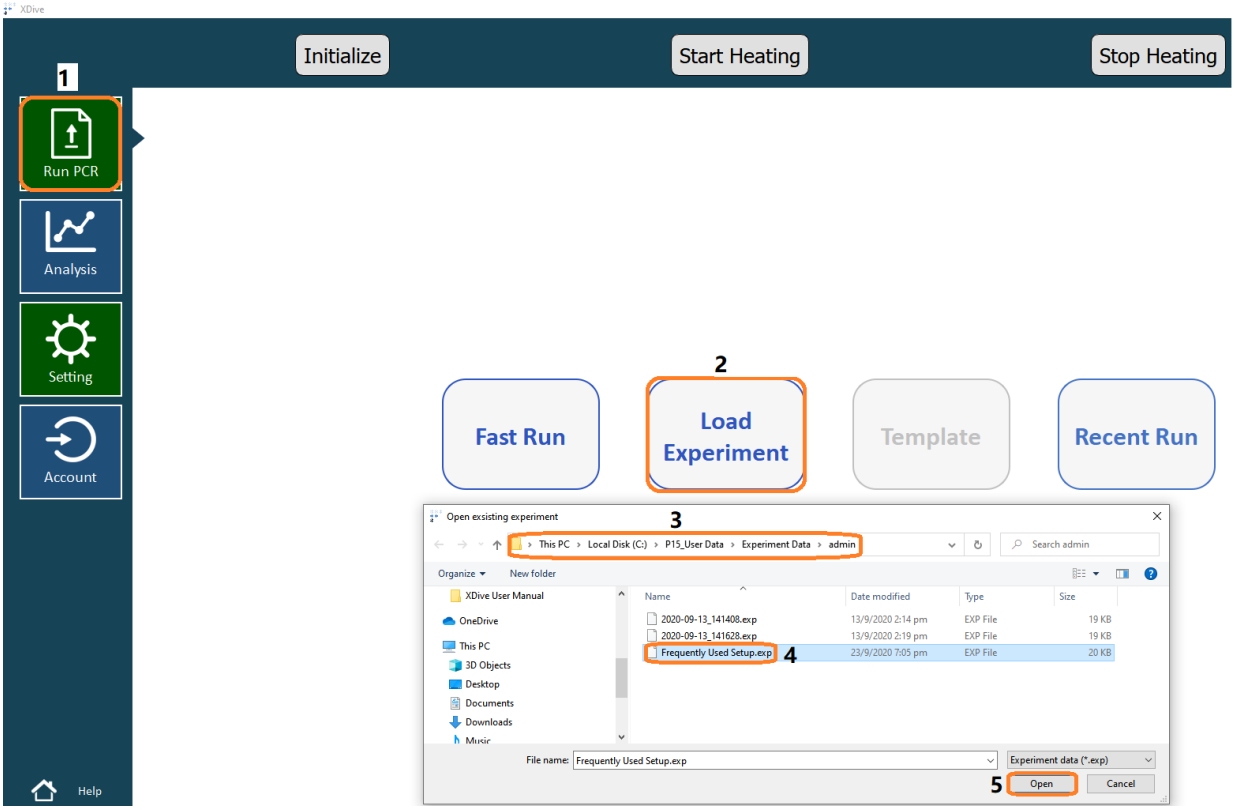
9.04.09d) Click "*Next*".



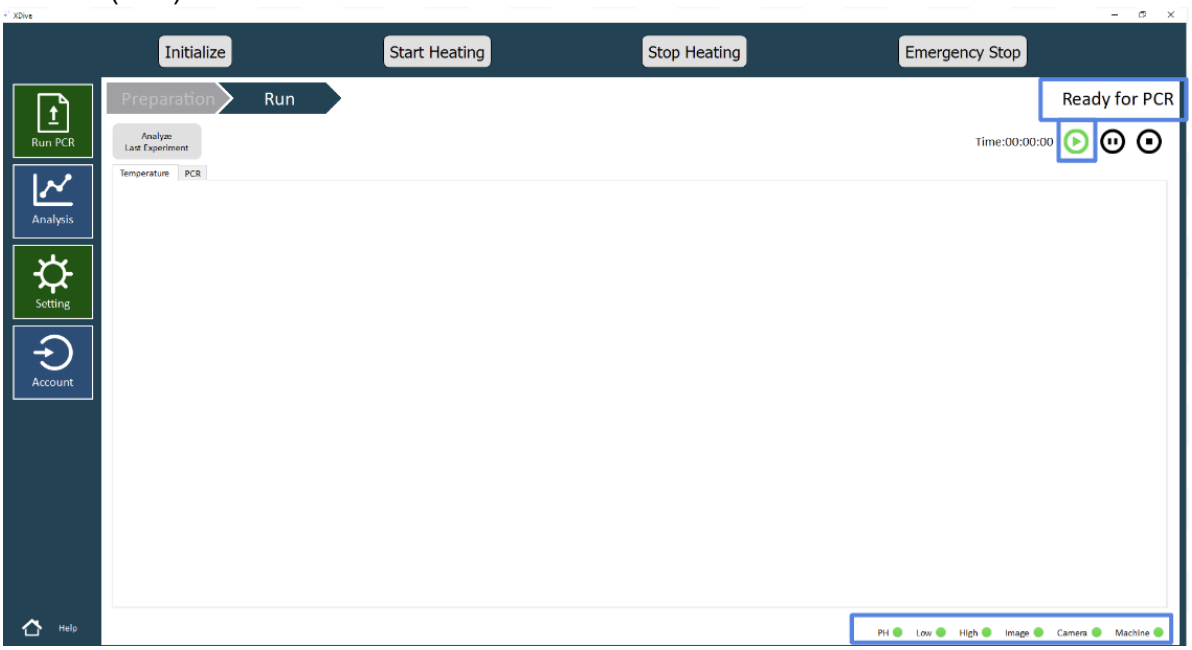
9.04.10) To save frequently used setup for future experiment:
On the lower right corner, click "*Save As*", then rename and browse for the directory of user's preference.



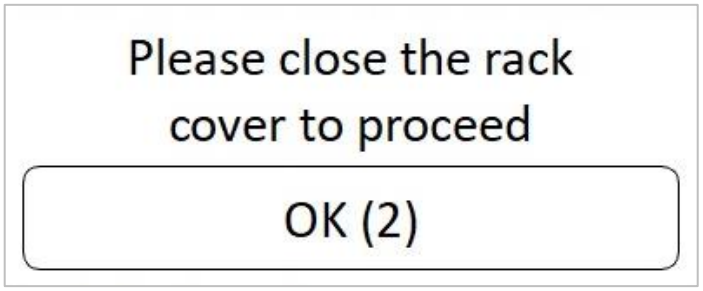
9.04.11) In the future, to load this experiment setup, user can click "*Run PCR*", then click "*Load Experiment*", and browse at the same directory for this saved experiment setup.



9.04.12) Once the “Ready for PCR” status is displayed, when sample(s) is loaded and ready, user can click the “▶” (Run) button to initiate the PCR Run.



Common message pop up for new users:



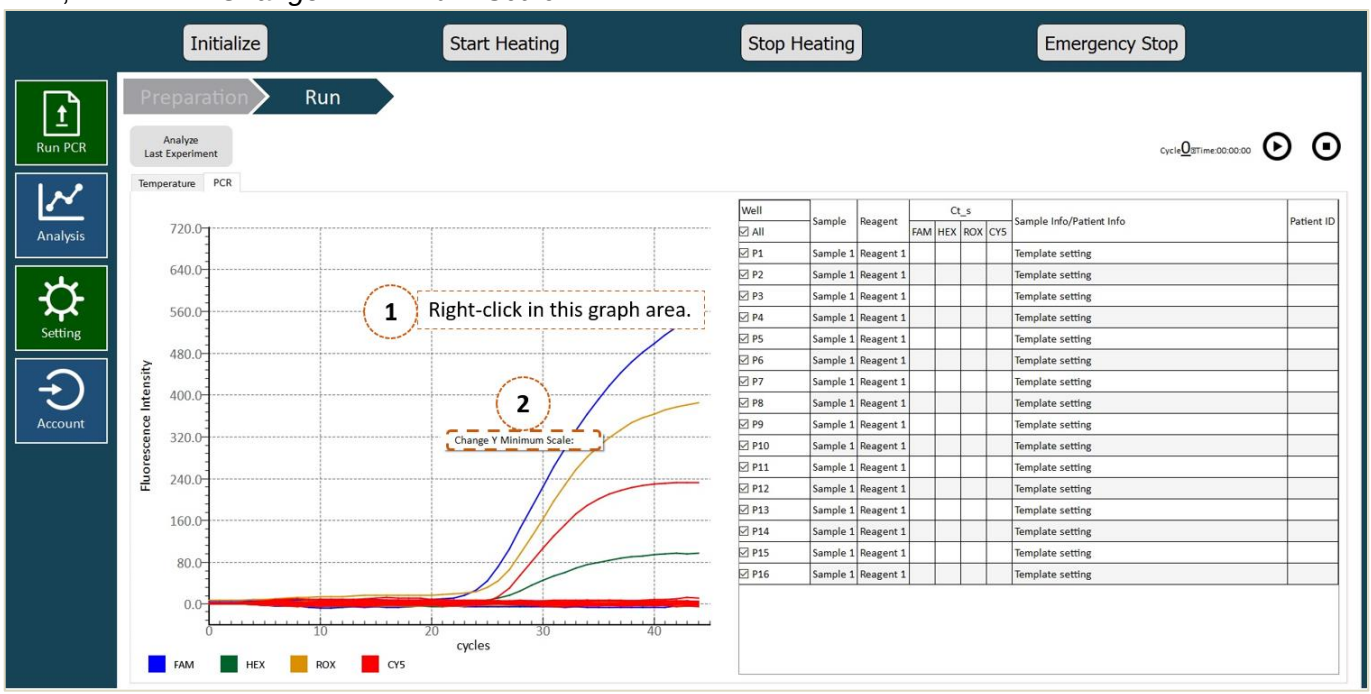
When the message above pops up, open the front door, unlatch the metal flap by raising the black hook, this will allow the sprint-loaded metal flap to go back to its pressing position.

9.04.13) By clicking the "*Temperature*" tab, the real-time temperature curve can be displayed.

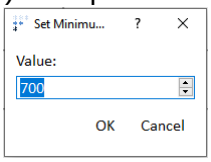
9.04.14) The "*PCR*" tab displays the progress of "*Fluorescence Intensity*".

9.04.15) User can selectively show / hide the curve of certain position by clicking on the correspondent checkbox(es).

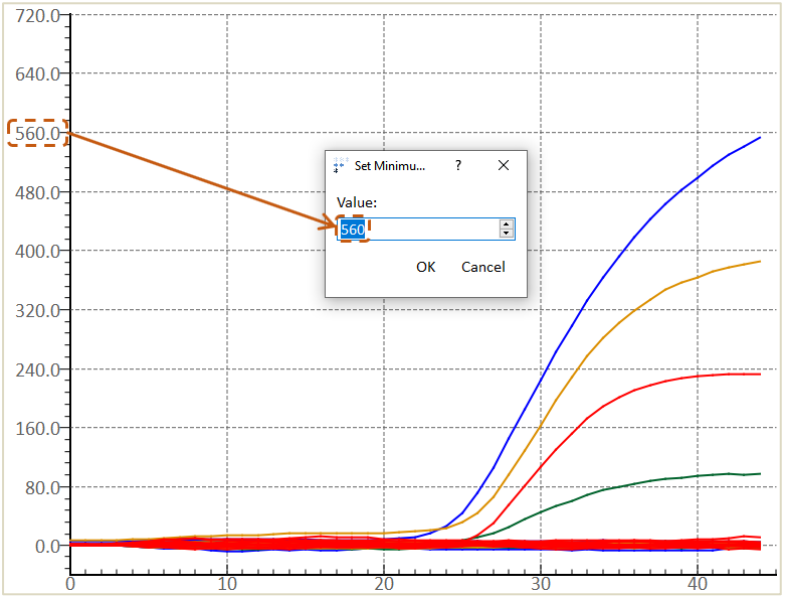
9.04.16) User can adjust the vertical scale of the Fluorescence Intensity graph by right-clicking in the curve area, then click "*Change Y Minimum Scale:"*



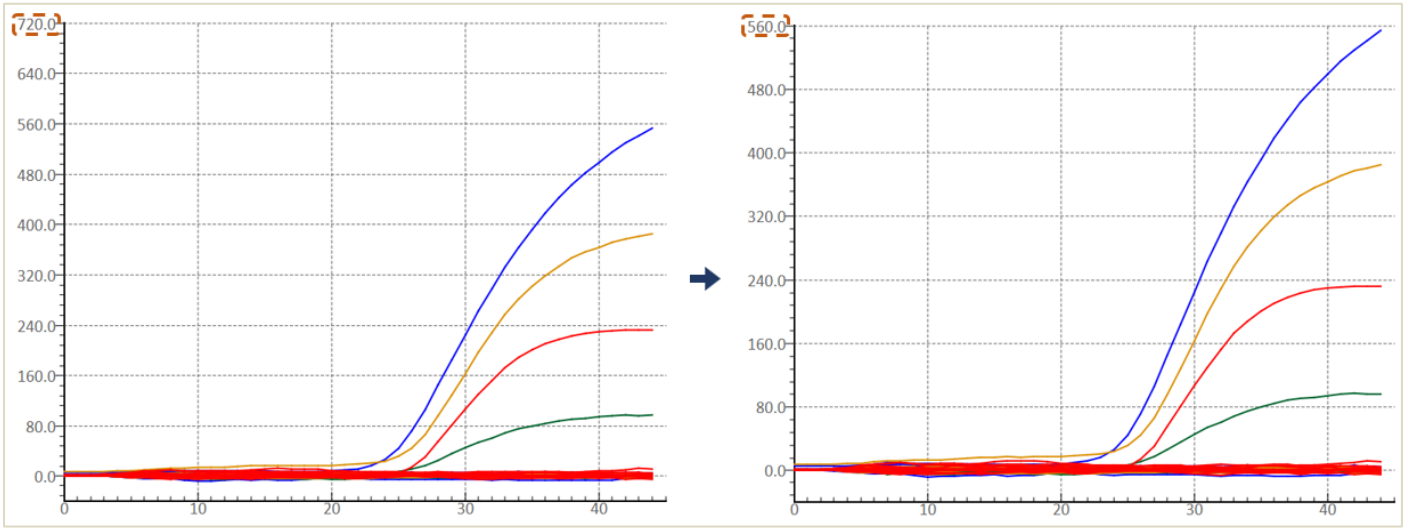
9.04.17) An input field will appear.



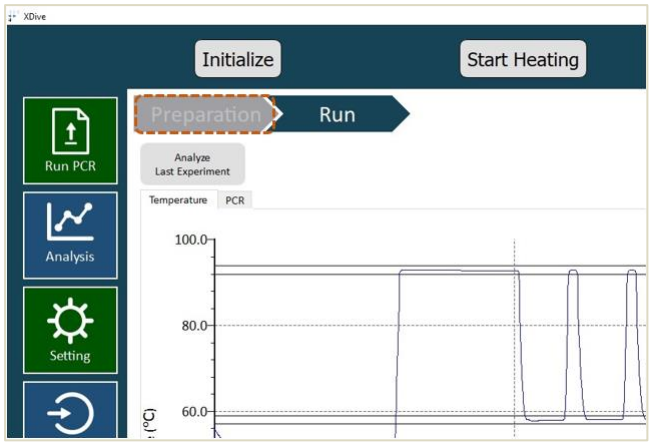
9.04.18) User can change the "*Y Minimum Scale*" value. In this case, for example, the number right above the highest curve line.



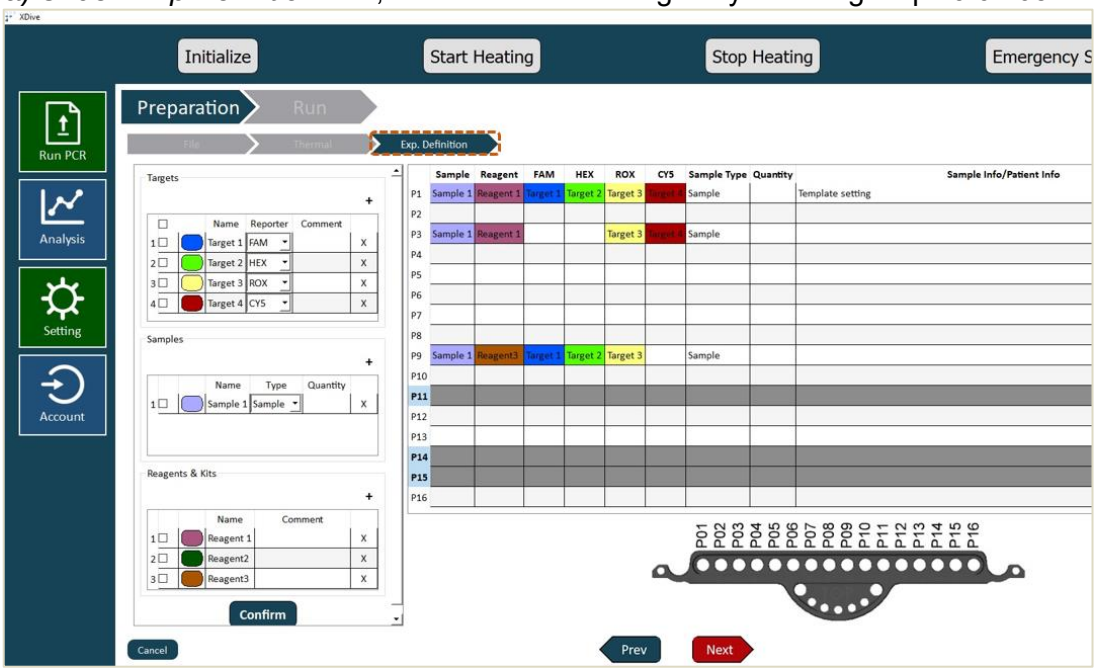
9.04.19) The curve will be stretched accordingly.



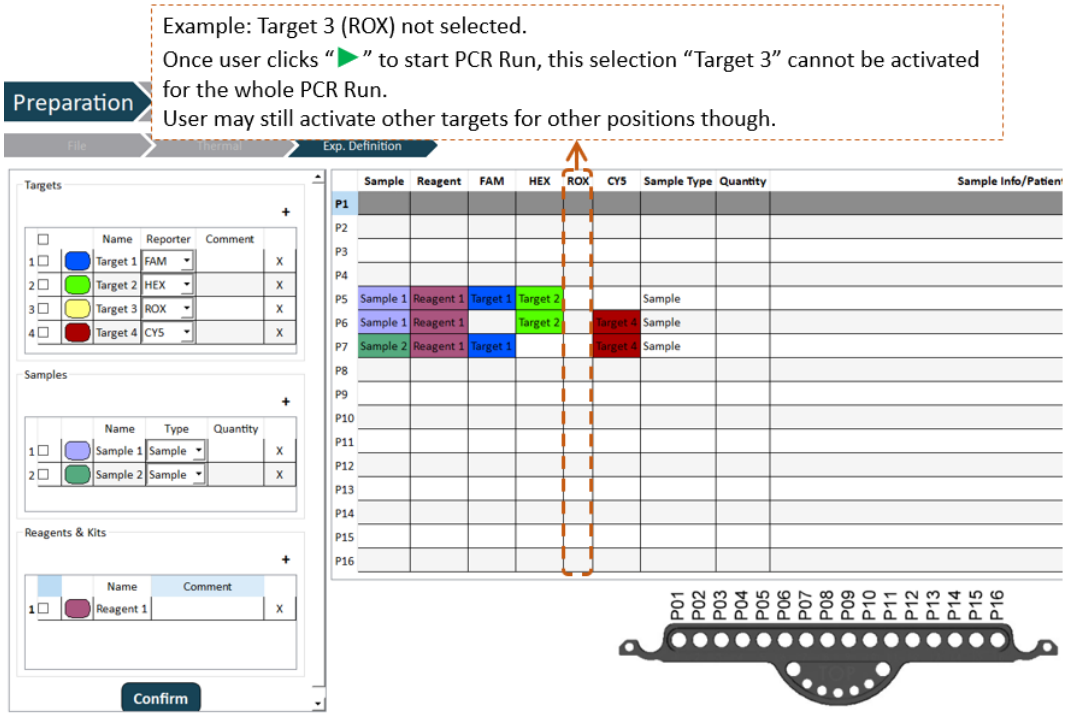
9.04.20) Before and during PCR Run, user can add or change selections under "*Exp. Definition*" by clicking "*Preparation*".



9.04.21a) Under "*Exp Definition*" tab, user can make changes by following steps 9.04.08a to 9.04.10.



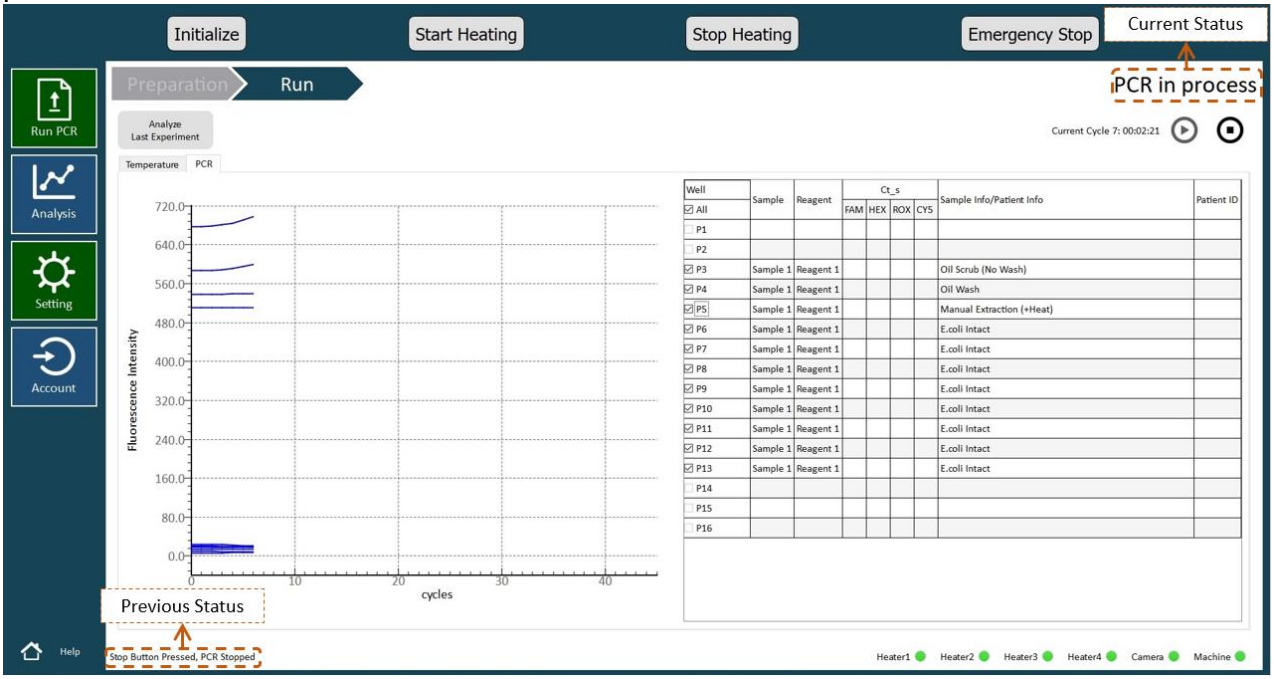
9.04.21b) After the PCR Run has started, the unselected target(s) can no longer be added during or after PCR Run.



Hence, user is suggested to carefully confirm each selection before clicking "▶" (Run) button.

9.04.22) After the PCR Run is completed, all these records will be fixed, and can no longer be edited.

9.04.23) The software displays the system's current status at the upper right corner while it displays the previous status at the lower left corner.

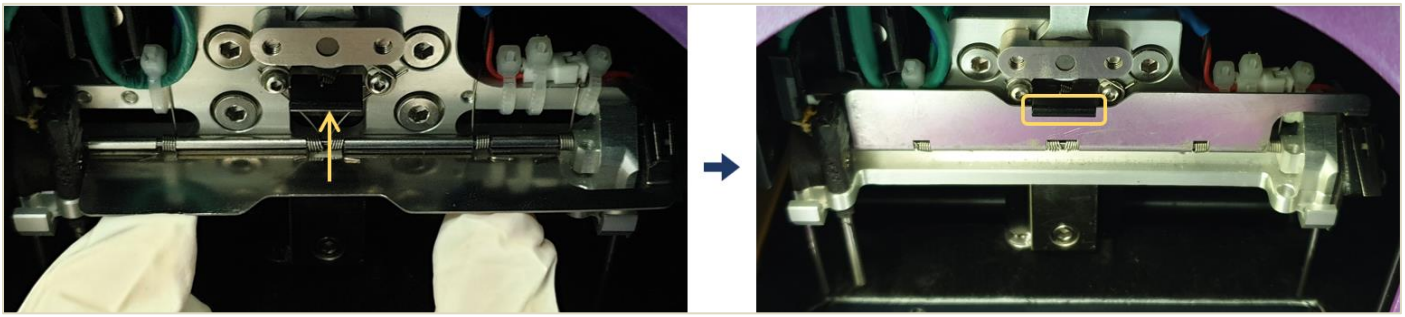


9.05 To Load / Unload Capillary That Contains Sample

9.05.01) Ensure that the machine's power is on.
If the sample holder mechanism is not at the required position, then on XDive software, click "Quick Control" => "Initialization".

9.05.02) Press once on the highlighted zone, the front door will be opened.

9.05.03) Flip up the metal flap till it is latched.
Remove the existing capillary tube holder if necessary.

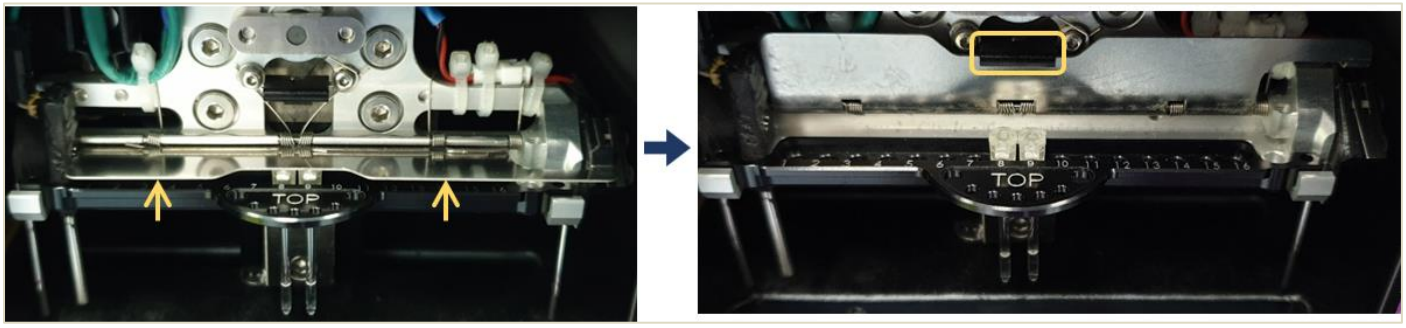


[1] Date of latest software update: 31-Mar-22

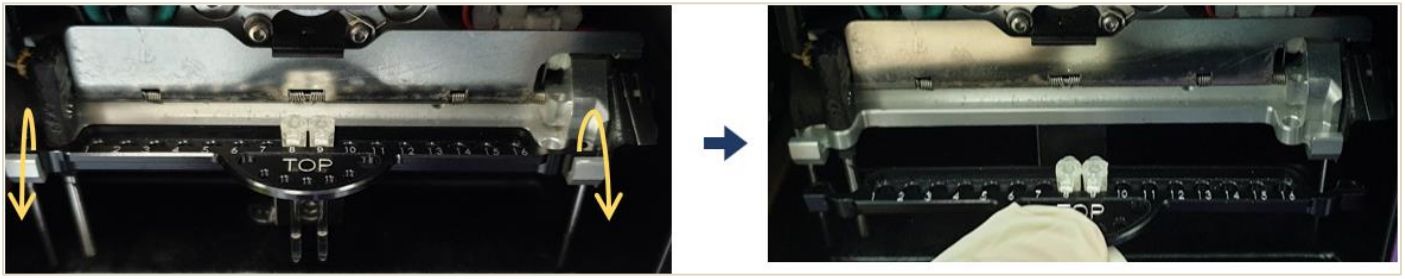
9.05.04) Load the capillary in Capillary Tube Holder onto the designated place.
(To find out how to dispense reagent into the XDive PCR Reaction Capillary Tube, please refer to the document "*Manual_The Use of XDive Deep Loading Tip*".)

9.05.05) Unlatch the metal flap by raising the black hook, this will allow the sprint-loaded metal flap to go back to its pressing position.

9.05.06) To unload capillary / rack, open the front door, then flip up the metal flap till it is latched.



9.05.07) Hold the holder's handle, raise it and take it out.



[1] Date of latest software update: 31-Mar-22

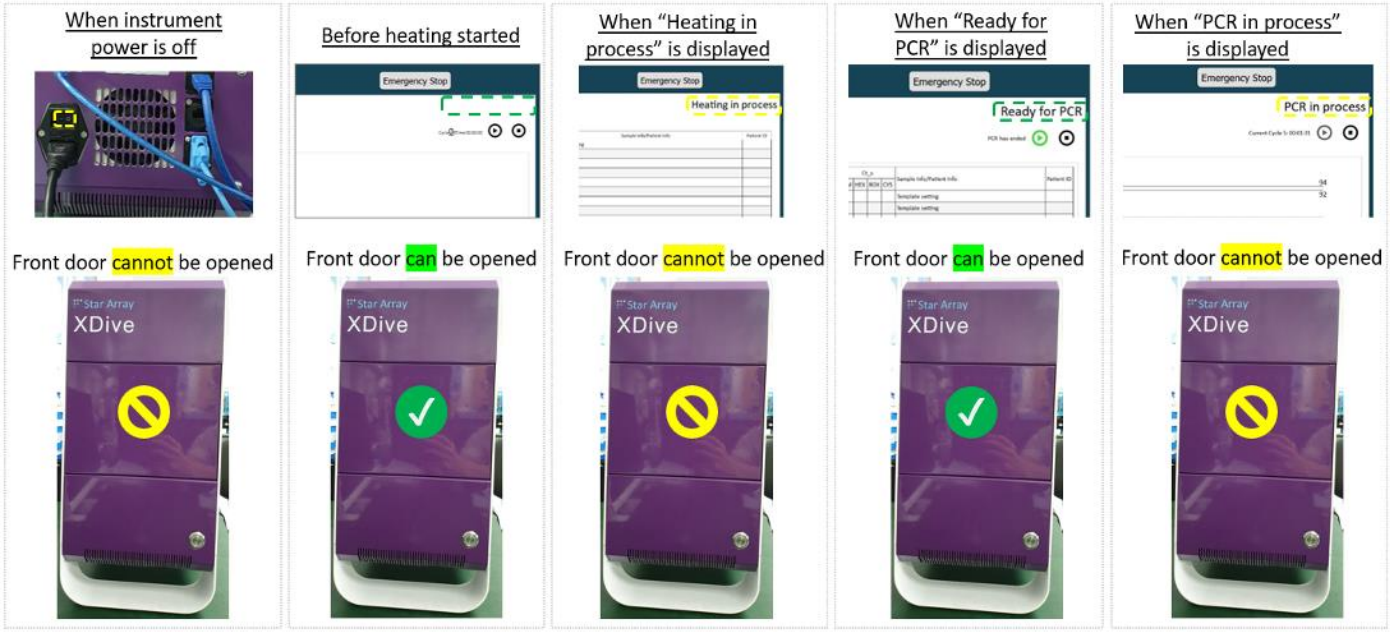
9.05.08) Close the front door, press it till you sense a light click.

9.05.09) The capillary tubes are to be used in pair.

If only one capillary tube is needed for experiment, then leave another empty.

Do not separate the capillary tube pair.

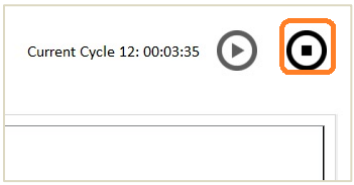
9.05.10) When “Heating in process” or “PCR in process” is displayed, the front door cannot be opened.

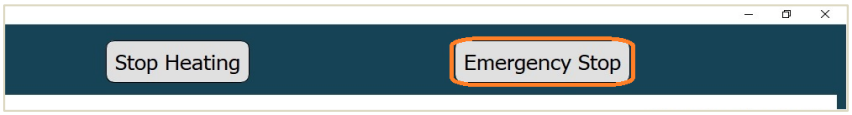


[1] Date of latest software update: 31-Mar-22

9.06 To abort a run

9.06.01) While a run is ongoing, user may abort the run by clicking either the "*Stop*" or the "*Emergency Stop*" button.

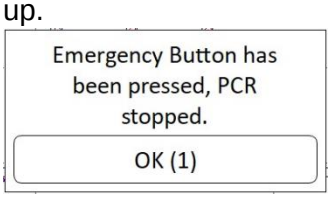




9.06.02) When prompted to confirm, select "*Yes*" to abort run.

9.06.03) Alternatively, user can also press the physical button under front door.

The PCR run will be stopped immediately without any warning message, and a reporting message will pop up.



[1] Date of latest software update: 31-Mar-22

9.06.04) To get the sample holder mechanism back in load / unload position, click "*Initialize*".

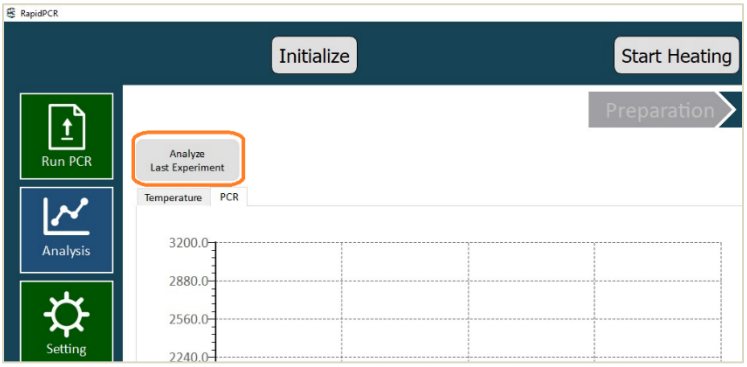
9.07 Disposal of Consumables & Reagents

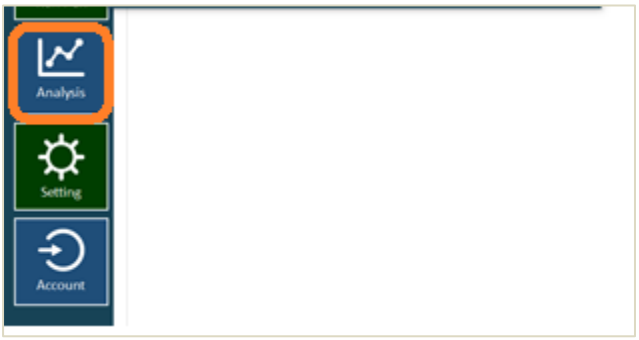
- Discard the capillaries into a solid waste box after use.
- Discard reagents and waste material according to local safety guidelines.
- Contact Star Array for disposal of the instrument or instrument parts.

9.08 Data Analysis

Note:
This Analysis program is only for user to analyze the experiment data of valid fresh reagent.
User may leave the reactor capillary that contains used reagent loaded in the XDive instrument for repeated PCR run, but the analysis of reused PCR reagent may not make sense.

9.08.01) After the PCR Run is finished, click "*Analyze Last Experiment*" or "*Analysis*".





Note: If the experiment data file is renamed, then the "*Analyze Last Experiment*" function will not be able to successfully load the renamed experiment data file. In this case, user can use the "Analysis" button to browse for the renamed data file.

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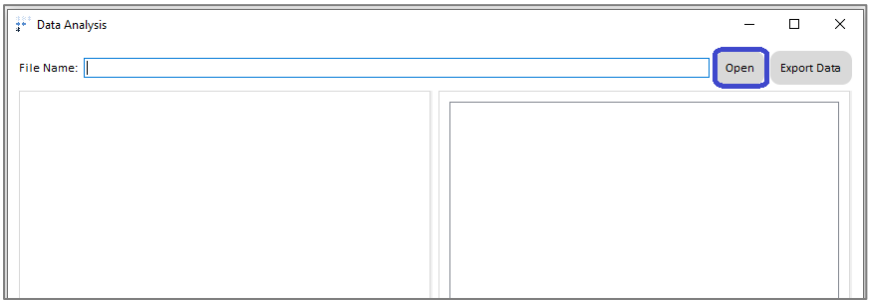
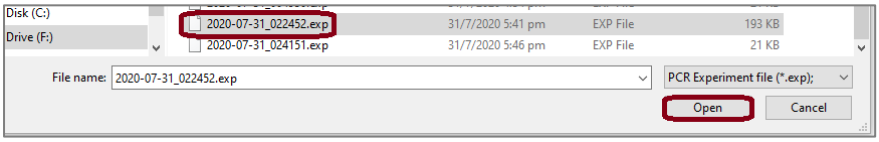
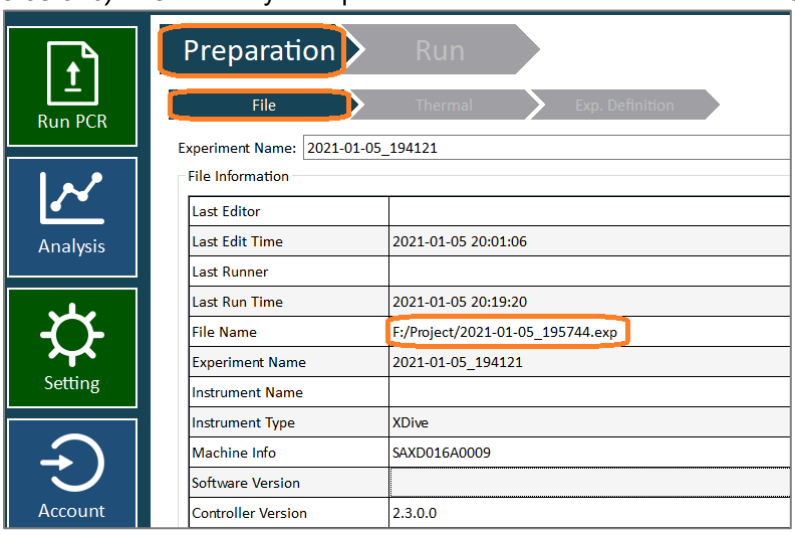
9.08.02a) If "*Analysis*" is clicked, then click "*Open*", then browse for the test data in *.exp* format, then click "*Open*".





9.08.02b) The directory of experiment data file can be traced at "*Preparation*" -> "*File*" -> "*File Name*".



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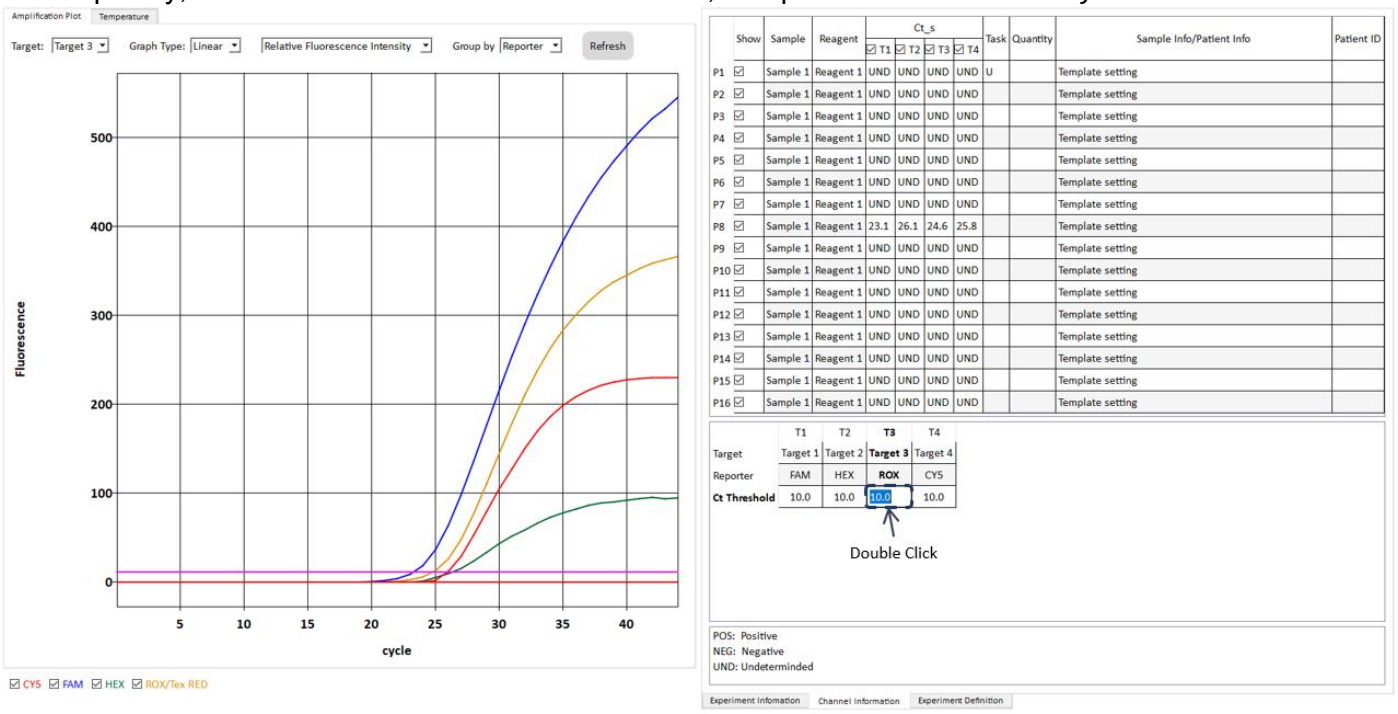
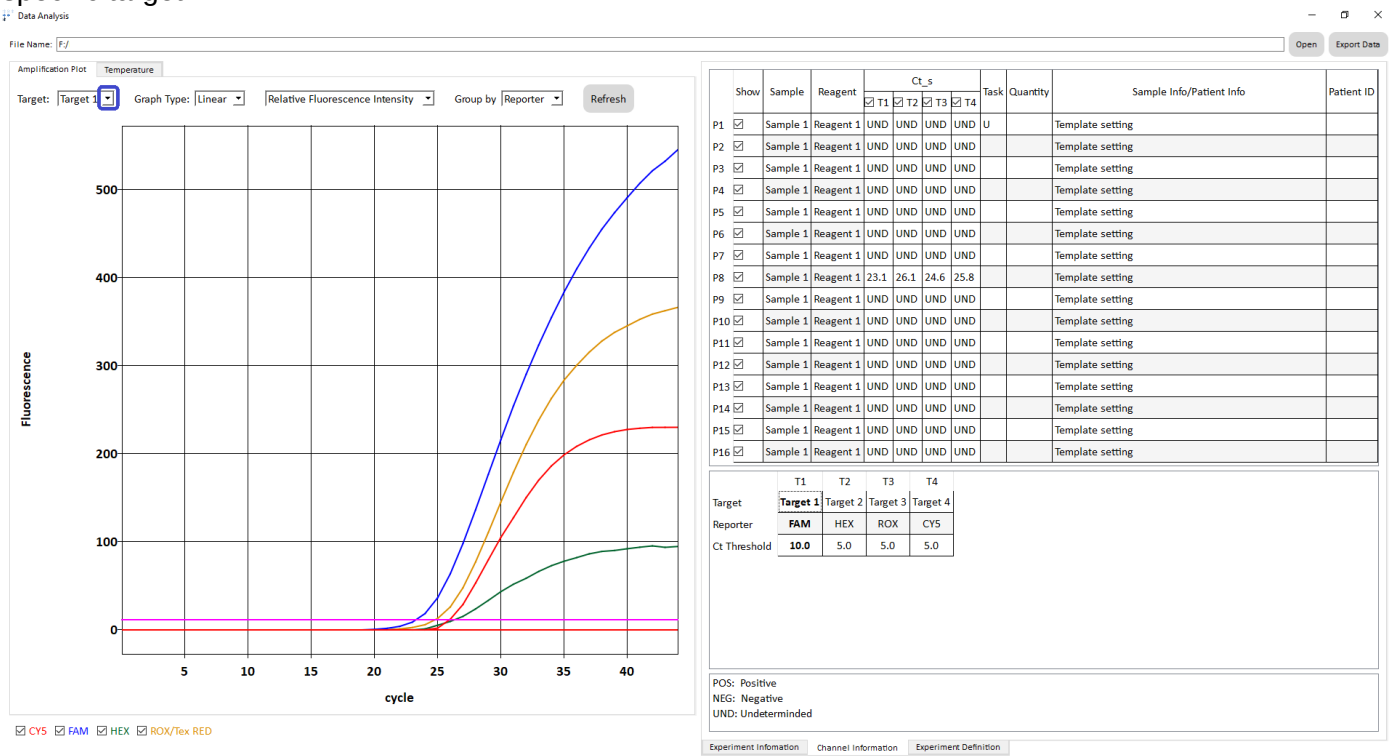
9.08.03a) To adjust the Ct Threshold value of specific target, double click on the number under its correspondent target. In the example below, under "*Target 3*".
Subsequently, enter a number that user intends to use, and press "*Enter*" on the keyboard.



9.08.03b) Alternatively, user can click the Target's drop-down menu to adjust the Ct Threshold value of specific target.



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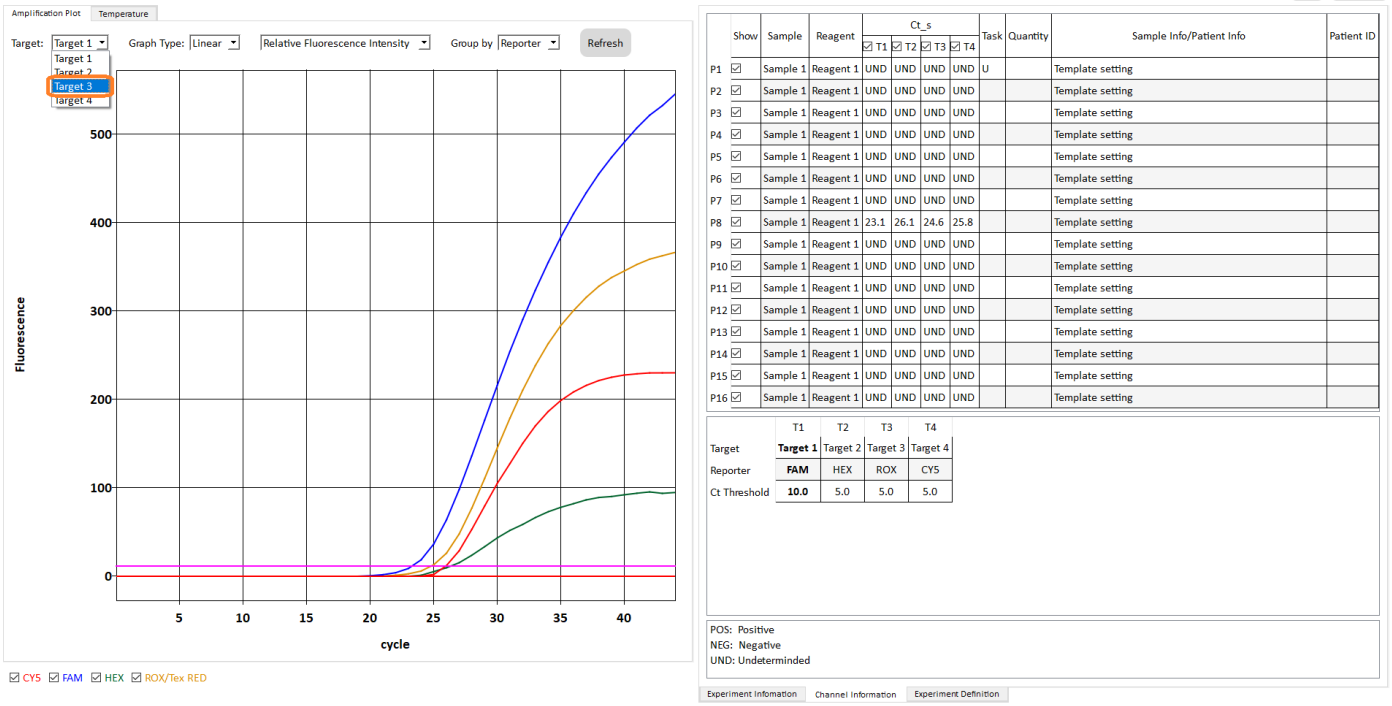
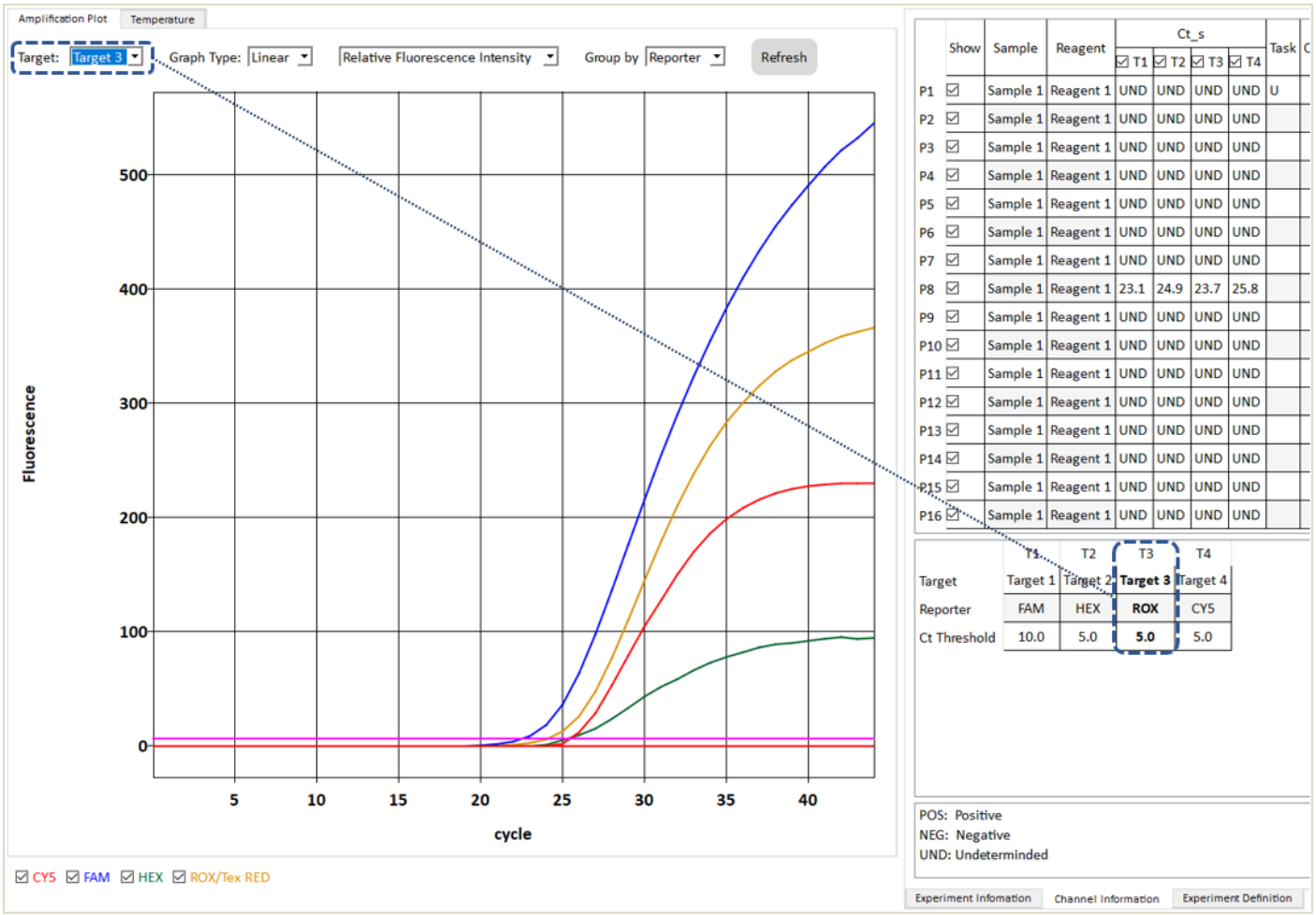
9.08.03c) For example, if user wants to adjust the Ct Threshold value of Target 3, user can select “Target 3” from the drop-down menu.



9.08.03d) Once selected, the text of “*T3*” column will be displayed as bold, and user can adjust its Ct Threshold value by entering the number in mind.



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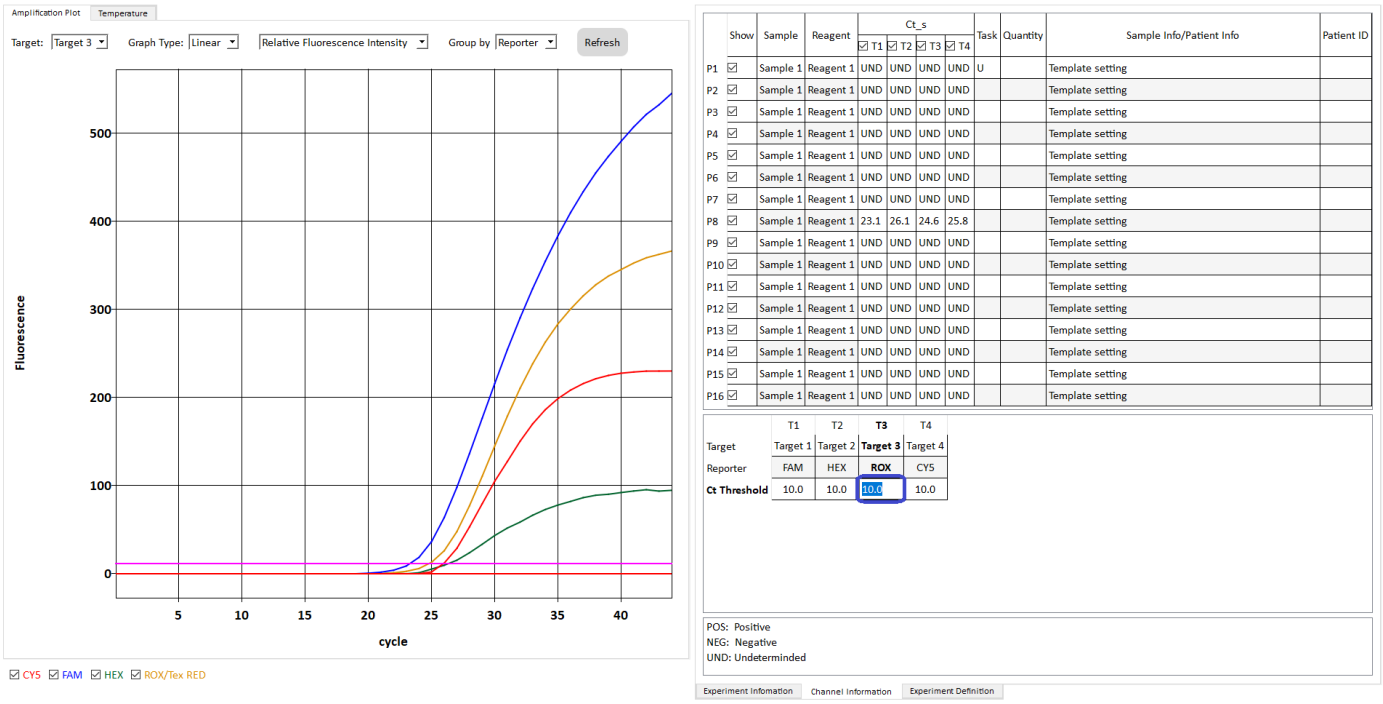
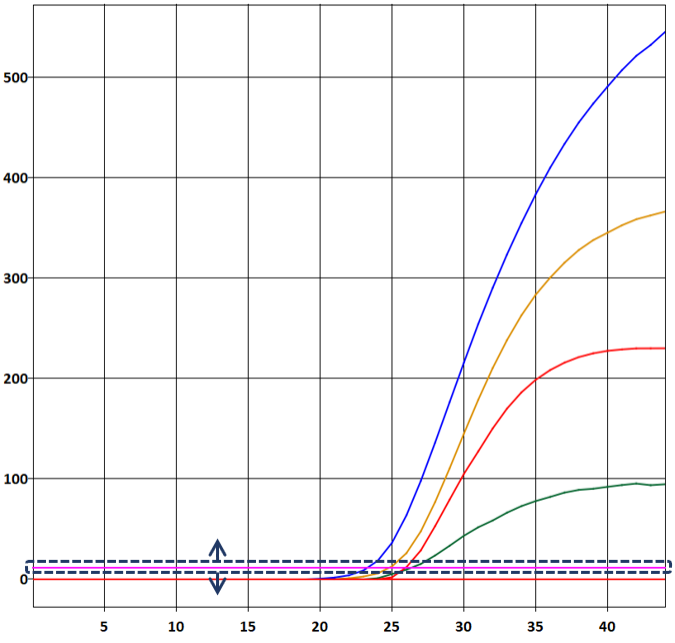


9.08.03e) Alternatively, user can also adjust the Ct Threshold level by left-clicking the pink line and drag it vertically.



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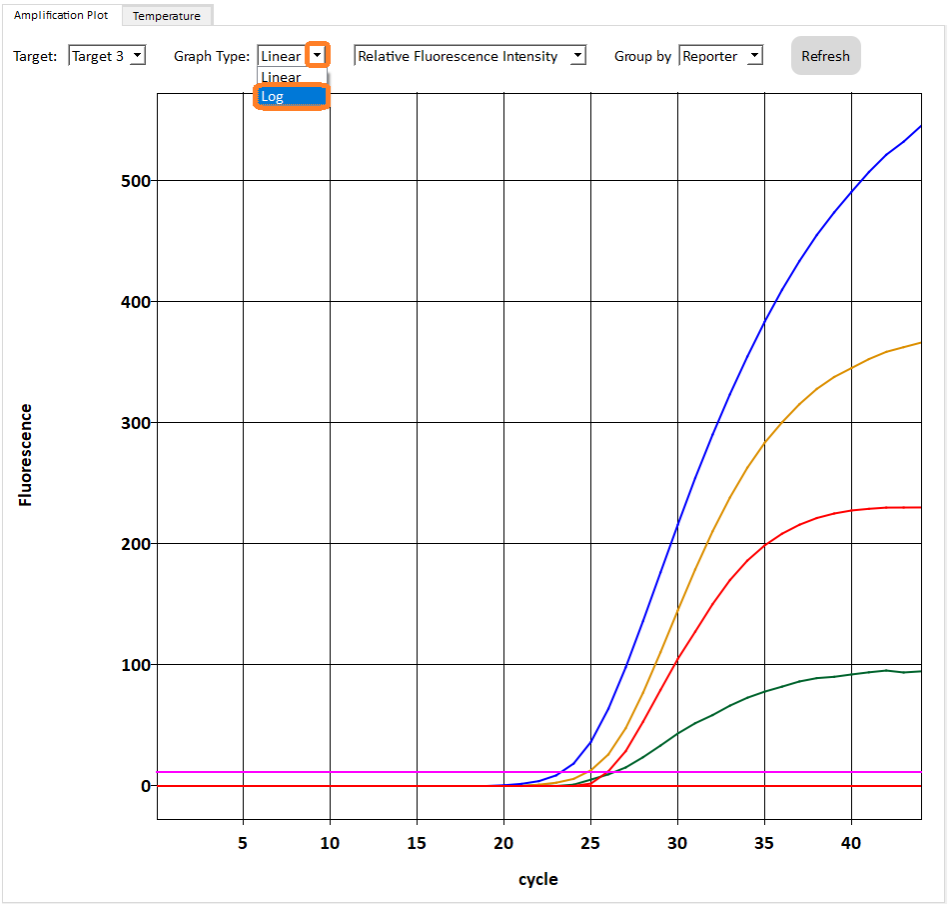
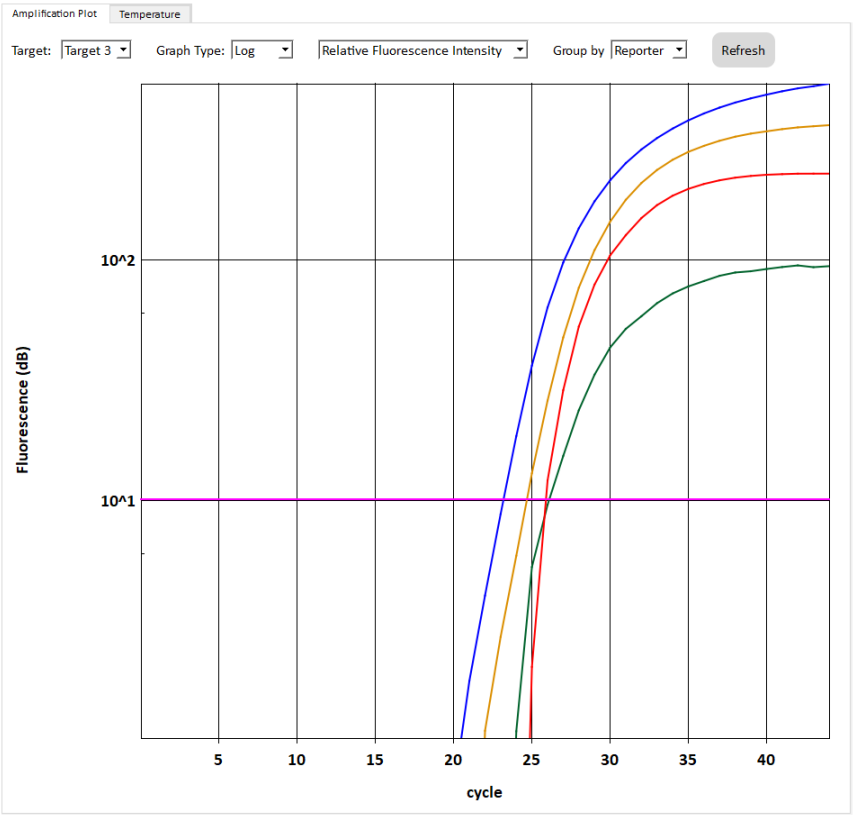
9.08.04) User can switch to the "*Log*" view by clicking on the drop-down menu besides "*Graph Type*", then select "*Log*".



The graph type will be displayed in "*Log*" view.



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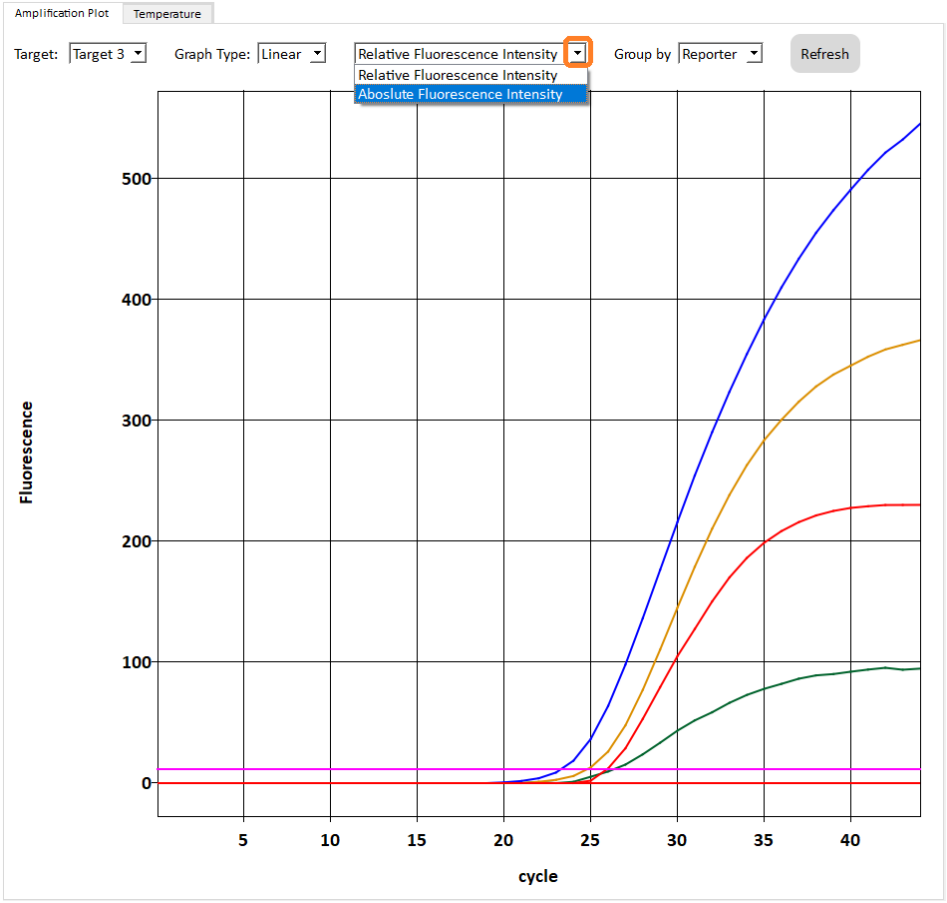
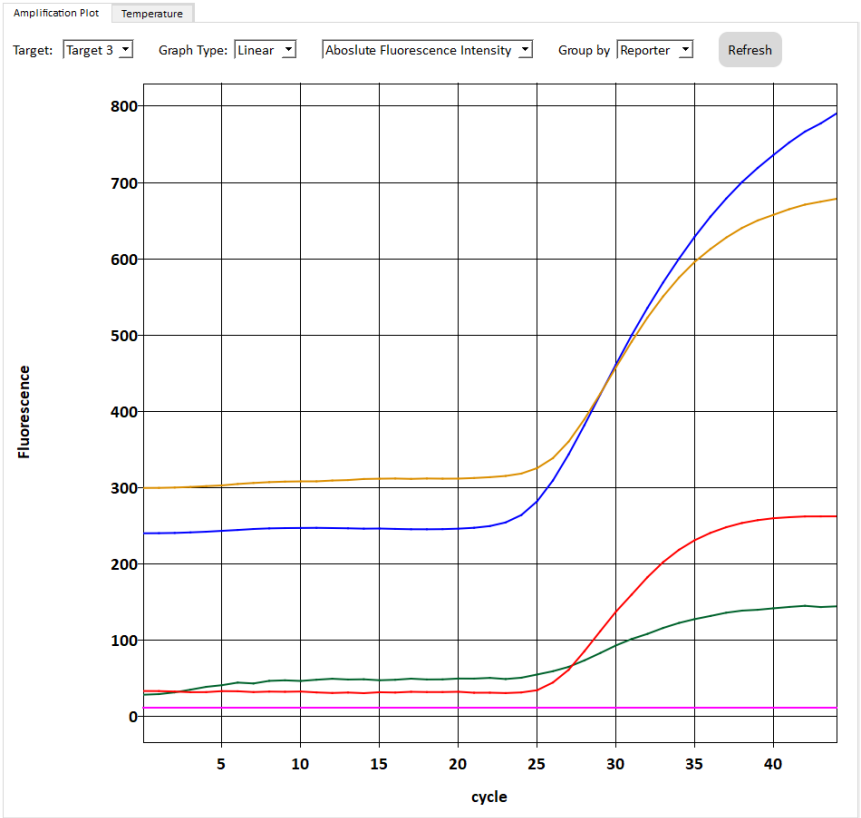
9.08.05) User can toggle between the "*Relative Fluorescence Intensity*" view (default, normalized) and the "*Absolute Fluorescence Intensity*" (unnormalized) by expanding the drop-down menu highlighted in the picture below.



The display will be switched to the "*Absolute Fluorescence Intensity*" view.



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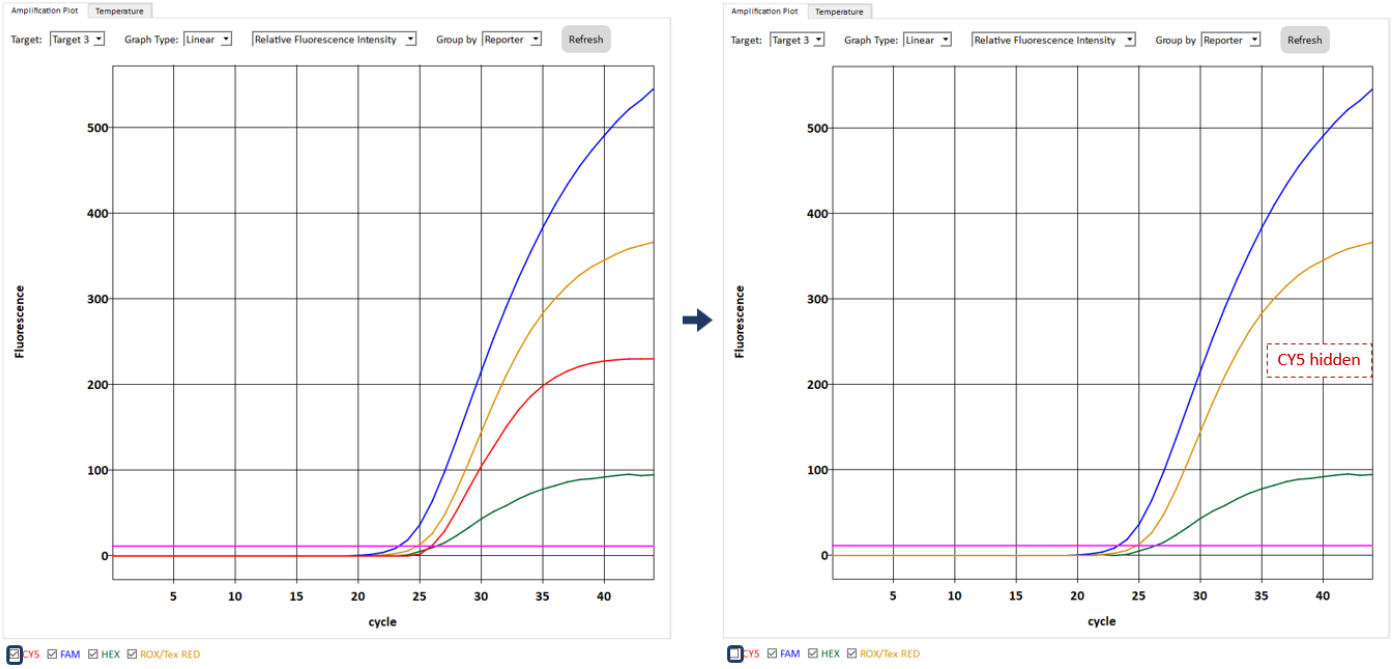
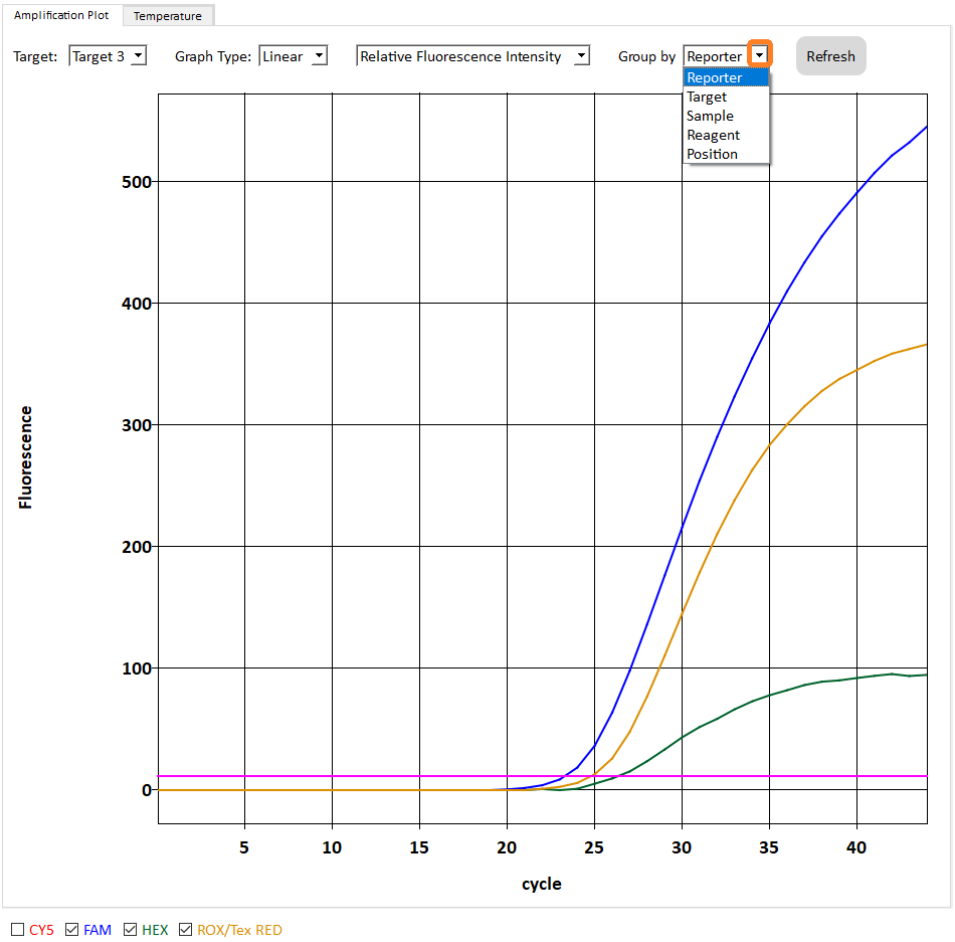
9.08.06a) The default "*Group by*" display is "*Reporter*". Now user can selectively show / hide certain group of "*Reporter*" by clicking on the checkbox(es).
In this case, for example, by unticking the "*CY5*" checkbox, the *CY5* curve is then hidden.



9.08.06b) User can also group the curves by the other aspects.



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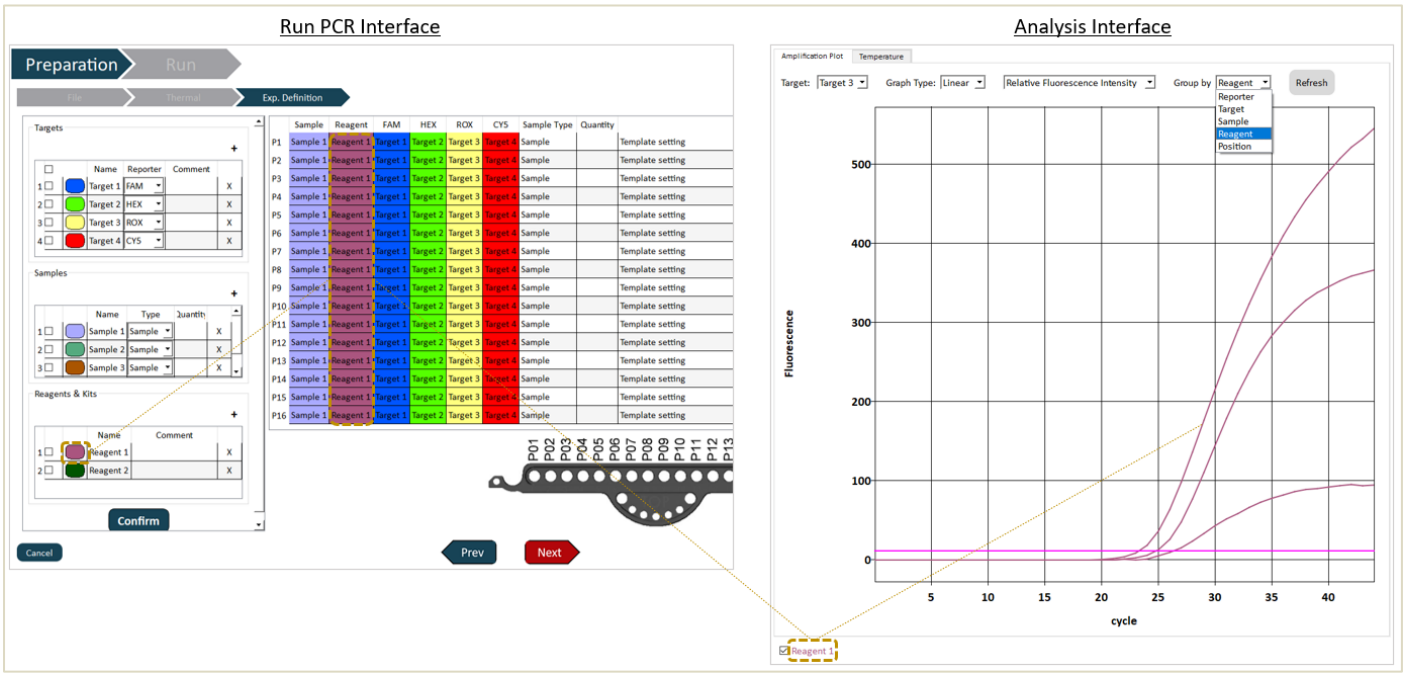
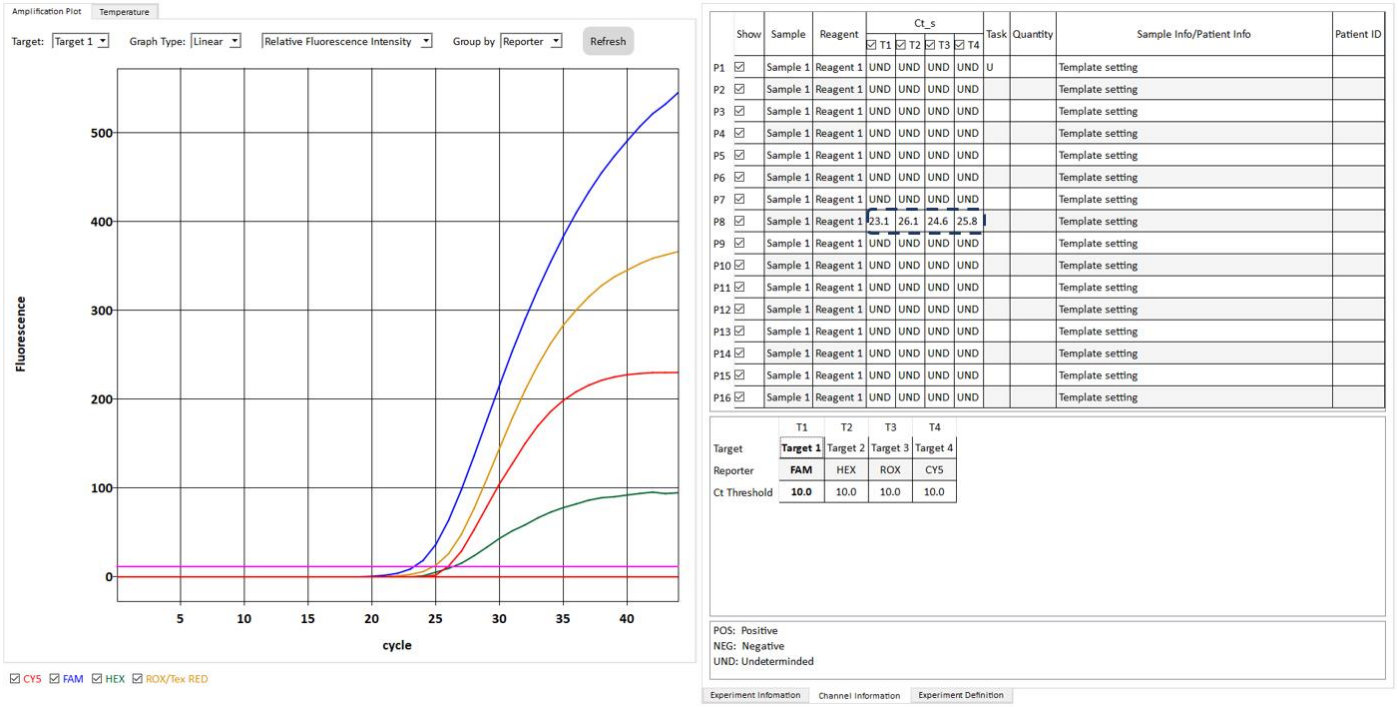
9.08.06c) The color displayed is defined when user setup the experiment. Take the color of "*Reagent 1*" as example:



9.08.07a) The Ct values are displayed under the "*Ct_s*" column.



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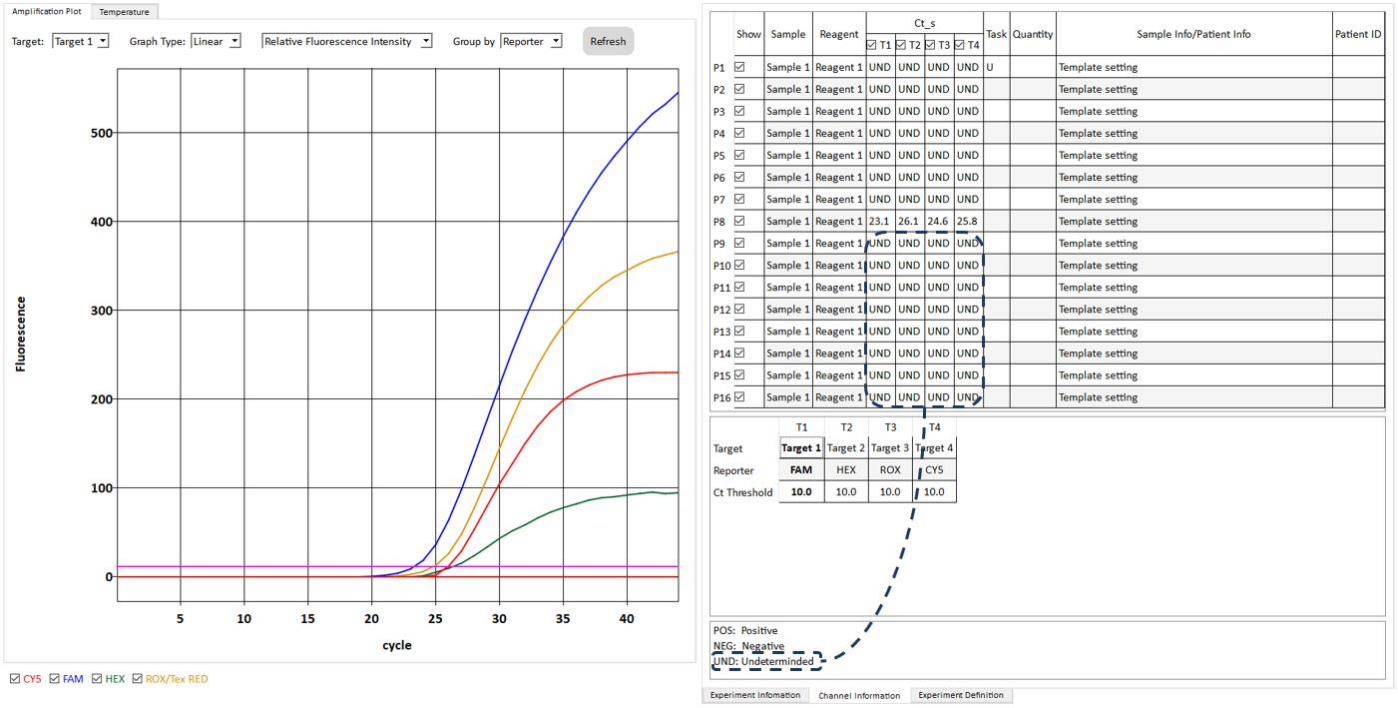
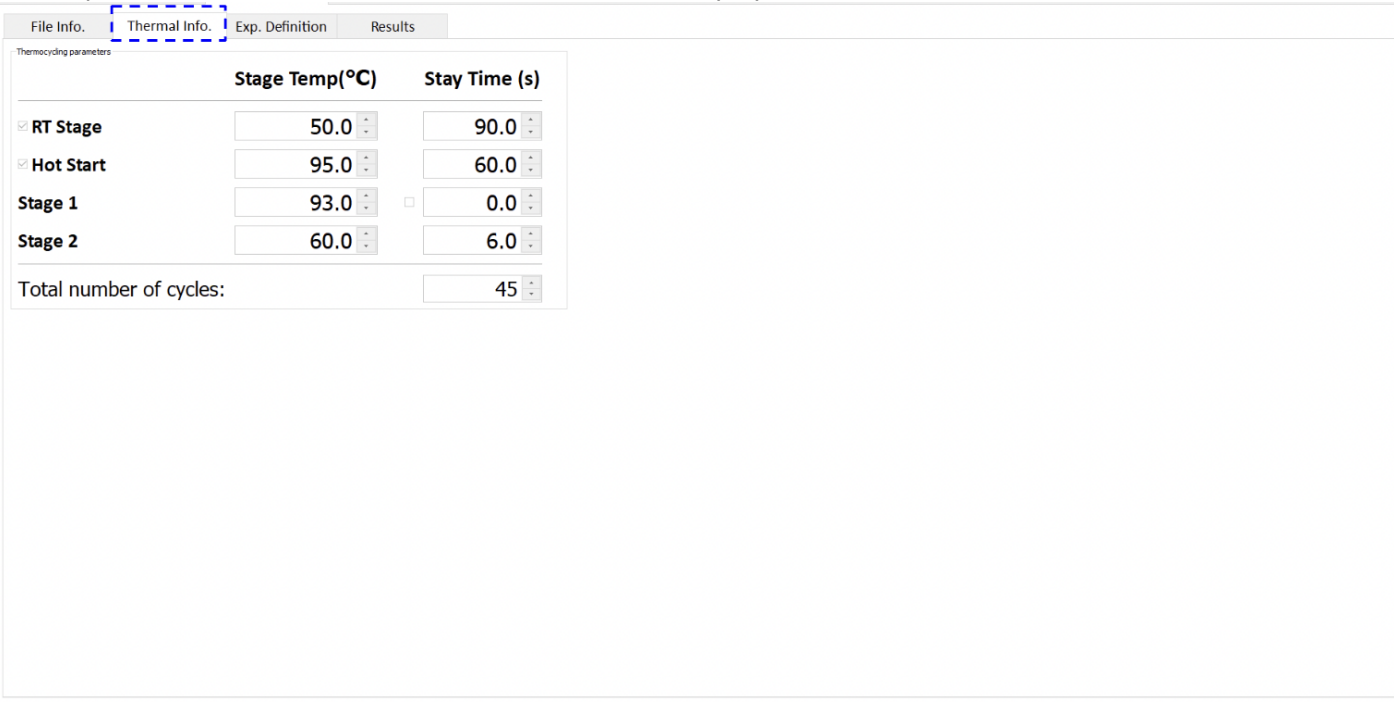
9.08.07b) Those "*UND*" means "*Undetermined*".



9.08.08) User can switch to the "*Thermal Info*" tab for review purpose.



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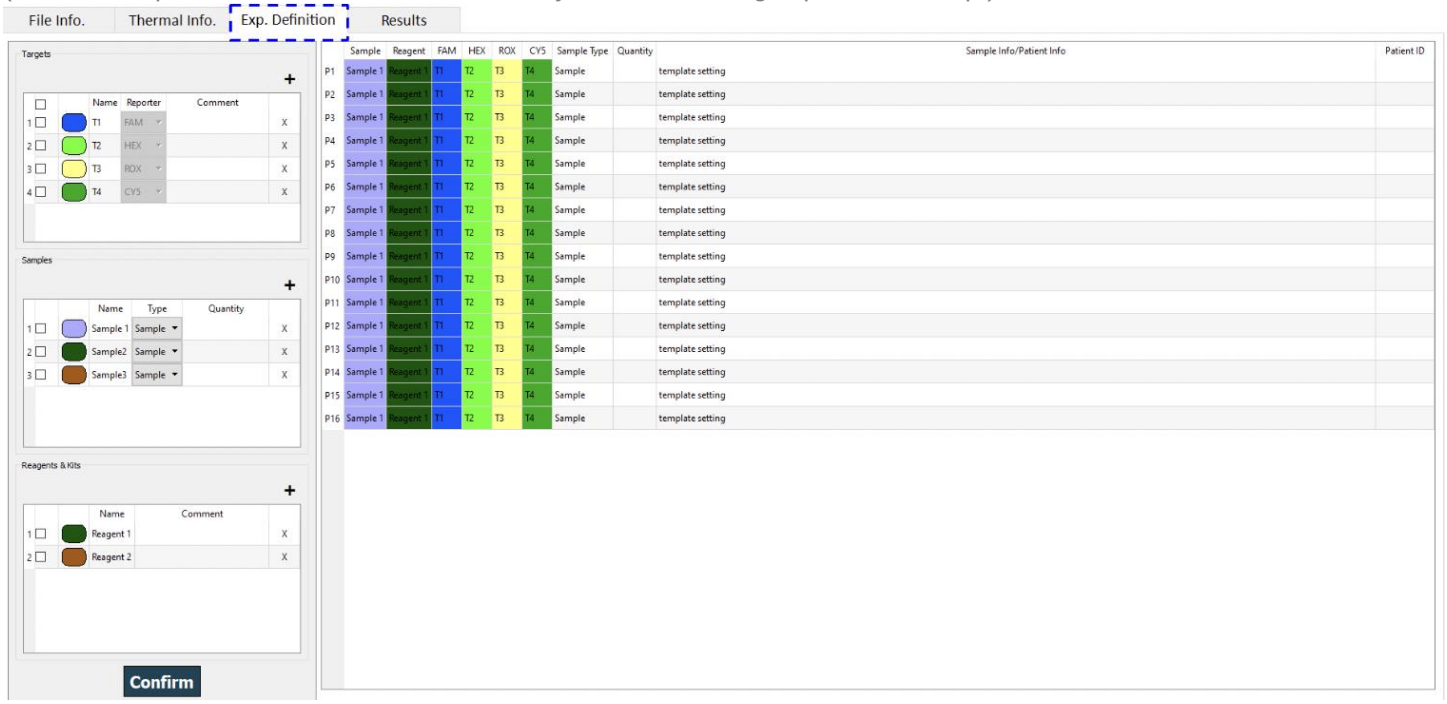
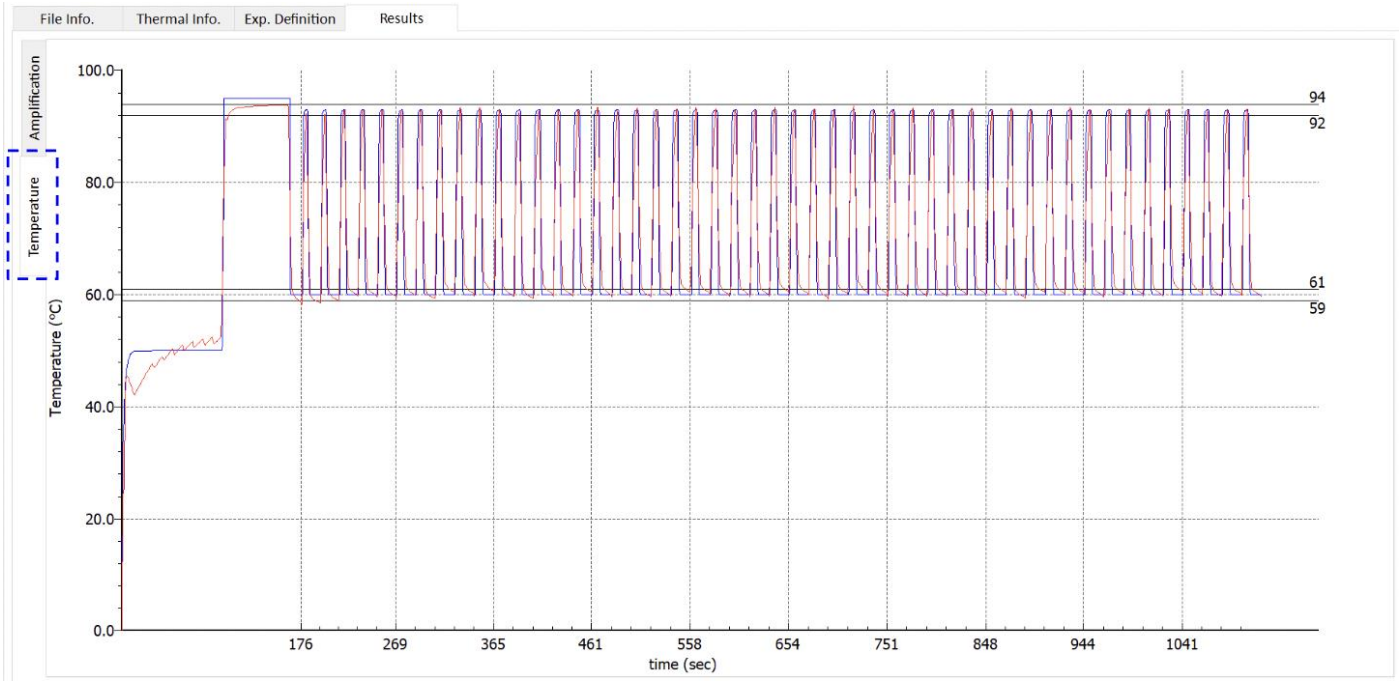
9.08.09) User can also switch to "*Exp. Definition*" tab to trace back sample information inserted during experiment setup.

(In this example below, the user did not add any comment during experiment setup.)



9.08.10) The "*Temperature*" tab records the temperature curve.



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10. Troubleshooting & Repair

Data derived from a run where a system message appeared should be reviewed carefully. If the validity of the results is doubtful, then repeat the run.

Issue	Type	Possible Cause	Solution
When user clicks "▶" (Run) button, message *"Rack cover is open, please close it before proceeding"* pops up.	Status Display	Metal flap is not at down position.	Unlatch the metal flap by raising the black hook, this will allow the sprint-loaded metal flap to go back to its pressing position. (Reference: Step 9.05.06) Then retry clicking "▶".
When user clicks "▶" (Run) button, message "*The tank is still heating, please wait." pops up.*	Notify	Tank temperature hasn't reached its target.	Wait till tank temperature reaches its target. (Reference: Step 9.04.08c) Then retry clicking "▶".
Under *"Exp. Definition"* tab, *w*hen user clicks any object (e.g. checkbox) but hasn't got the expected response.	Interface Response	N/A	Click "Save As", define directory & file name for this experiment, then retry clicking objects under "Exp. Definition" tab. (Reference: Step 9.04.08d)

For any issue not mentioned above, please contact Star Array via phone number or email address mentioned in section 1.

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11. Maintenance

11.01 General Cleaning

Clean the housing of the XDive Machine with a mild commercial detergent. If necessary, use 70% ethanol for disinfection of the instrument housing.

11.02 Special Cleaning

As with all potentially bio-hazardous specimens, universal safety precautions shall be taken when handling and processing samples. Spills shall be immediately disinfected with an appropriate disinfectant solution to avoid spreading contamination to laboratory personnel or equipment.

11.03 Preventive Maintenance

Preventive maintenance of XDive Machine shall be performed by Star Array authorized personnel.

12. Spare Parts

- Star Array glass capillary rack.
- Star Array temperature calibration sensor.
- Star Array thermal tank and thermal tank rod.
- Beads.
- Limit Switch.

13. Additional Equipment Required

The following additional equipment is required to perform real-time PCR assays with the XDive Machine System:

- Star Array centrifuge containing a rotor and two holders for glass capillaries.
- Nuclease-free, aerosol-resistant pipette tips.
- Pipettes with disposable, positive-displacement tips.
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions.

<End of User Manual>

[1] Date of latest software update: 31-Mar-22