



AMPIPROBE[®] SARS-CoV-2 Test System

INSTRUCTIONS FOR USE

Multiplex real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2

ENZ-GEN216-0960 (AMPIXTRACT[™] SARS-CoV-2 Extraction, 10 x 96 samples)

ENZ-GEN216-0096 (AMPIXTRACT[™] SARS-CoV-2 Extraction, 1 x 96 samples)

ENZ-GEN215-0096 (AMPIPROBE[®] SARS-CoV-2 Assay)

ENZ-GEN218-0004 (AMPIPROBE[®] SARS-CoV-2 Controls)

For the latest product information, including support documentation, visit us online:

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IVD

For *in vitro* diagnostic use

For use under Emergency Use Authorization (EUA) only | Rx only



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The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instruments.

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INTENDED USE

The AMPIPROBE® SARS-CoV-2 Test System is a multiplex assay system based on real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) collected 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, that meet requirements to perform high complexity tests.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to five individual upper respiratory swab specimens (such as nasal, mid-turbinate, nasopharyngeal or oropharyngeal swabs) that were collected in individual vials containing transport media from individuals suspected of COVID-19 by their health care provider. Negative results from pooled testing should not be treated as definitive. If patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive, inconclusive, or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory 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 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 AMPIPROBE® SARS-CoV-2 Test System is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The AMPIPROBE® SARS-CoV-2 Test System is only for use under the Food and Drug Administration's Emergency Use Authorization.

PRODUCT DESCRIPTION

The AMPIPROBE® SARS-CoV-2 Test System includes the following components:

- AMPIXTRACT™ SARS-CoV-2 Extraction kit
- AMPIPROBE® SARS-CoV-2 Assay kit
- AMPIPROBE® SARS-CoV-2 Controls kit

The AMPIXTRACT™ SARS-CoV-2 Extraction kit is available in two sizes: 1 x 96 sample extractions; and 10 x 96 sample extractions. The kit utilizes magnetic-particle technology for isolation and purification of nucleic acids from upper respiratory specimens.

The AMPIPROBE® SARS-CoV-2 Assay kit is a multiplexed real-time RT-PCR assay that contains two primer and probe sets to detect two regions in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. RNA isolated from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems QuantStudio® 5 (QS5) instrument with software version 1.5.1. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM, HEX, and Quasar 670) to separate from the quencher dye (BHQ1 or BHQ2), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the thermocycler. Each AMPIPROBE® SARS-CoV-2 Assay kit provides reagents for 96 assays.

The AMPIPROBE® SARS-CoV-2 Controls kit contains a positive RNA control that is specific to the SARS-CoV-2 genomic regions targeted by the assay as well as human RNase P, a negative RNA control that contains target to human RNase P, and a no-template control (NTC). Each kit provides control reagents for 4 runs.

KIT CONTENTS AND STORAGE

Store all unopened reagents and controls as recommended in the following tables. When stored properly, the reagents are stable until the expiration date indicated on the labels. Care must be taken to limit the number of freeze-thaw cycles for frozen reagents to no more than 3 times.

AMPIXTRACT™ SARS-CoV-2 Extraction (ENZ-GEN216-0960)^[a]			
Packs	Components	Quantity	Storage
AMPIXTRACT™ Wash Reagent Pack (Part No. ENZ-GEN219-0960) ^[a]	Wash Reagent	10 x 50 mL	RT (20-25°C)
AMPIXTRACT™ Lysis Reagent Pack (Part No. ENZ-GEN220-0960) ^[a]	Lysis Reagent	10 x 80 mL	RT (20-25°C)
AMPIXTRACT™ EMP Pack (Part No. ENZ-GEN221-0960) ^[a]	Elution Buffer	10 x 12 mL	2-8°C
	Magnetic Beads	10 x 5 mL	2-8°C
	PK Dilution Buffer	10 x 9 mL	2-8°C
AMPIXTRACT™ Stabilizer Reagent Pack (Part No. ENZ-GEN222-0960) ^[a]	Stabilizer	20 x 900 µL	-20°C
AMPIXTRACT™ Proteinase Enzyme Pack (Part No. ENZ-GEN223-0960) ^[a]	Proteinase	10 x 350 µL	-20°C
AMPIXTRACT™ Carrier RNA Pack (Part No. ENZ-GEN224-0960) ^[a]	Carrier RNA	10 x 1.2 mL	-20°C

^[a] This kit can be ordered as a stand-alone product.

AMPIXTRACT™ SARS-CoV-2 Extraction (ENZ-GEN216-0096)^[a]			
Components		Quantity	Storage
AMPIXTRACT™ Wash Reagent		1 x 50 mL	RT (20-25°C)
AMPIXTRACT™ Lysis Reagent		1 x 80 mL	RT (20-25°C)
AMPIXTRACT™ Elution Buffer		1 x 12 mL	2-8°C
AMPIXTRACT™ Magnetic Beads		1 x 5 mL	2-8°C
AMPIXTRACT™ PK Dilution Buffer		1 x 9 mL	2-8°C
SPC Pack	AMPIXTRACT™ Stabilizer	2 x 900 µL	-20°C
	AMPIXTRACT™ Proteinase	1 x 350 µL	-20°C
	AMPIXTRACT™ Carrier RNA	1 x 1.2 mL	-20°C

^[a] This kit can be ordered as a stand-alone product.

AMPIPROBE® SARS-CoV-2 Assay (ENZ-GEN215-0096)^[a]		
Components	Quantity	Storage
AMPIPROBE® RT-PCR Buffer	1.3 mL	-20°C
AMPIPROBE® SARS-CoV-2 Primer/Probe Mix	260 µL	-20°C
AMPIPROBE® RT-PCR Enzyme Mix	130 µL	-20°C
Nuclease-free Water	1 mL	-20°C

^[a] This kit can be ordered as a stand-alone product.

AMPIPROBE® SARS-CoV-2 Controls (ENZ-GEN218-0004)^[a]		
Components	Quantity	Storage
AMPIPROBE® SARS-CoV-2 Positive Control	100 µL	-80°C
AMPIPROBE® SARS-CoV-2 Negative Control	100 µL	-80°C
No Template Control (NTC)	100 µL	-80°C

^[a] This kit can be ordered as a stand-alone product.

REQUIRED MATERIALS NOT PROVIDED

1. Components Required But Not Included with the AMPIPROBE® SARS-CoV-2 Test System for automated extraction and PCR set-up using the GENFLEX® platform (to be supplied by the user)

Instruments and Equipment

- a. 200 mL Trough (Hamilton 56695-01)
- b. 20 mL Trough (Hamilton 96424-02)
- c. Plastic waste chute (Hamilton 185319)
- d. Waste container for hazardous waste (Hamilton 281520)
- e. Biohazard plastic liner for waste container (Hamilton 53686-01)
- f. Vortex mixer
- g. Microcentrifuge
- h. GENFLEX® platform V1.0 (Catalog number: ENZ-GEN-FLX) which consists of 2 components:
 - Hamilton Microlab Star (Hamilton, catalog number: 49000-60: GENFLEX® STAR) which is a liquid handler used for automated extraction and PCR set up and is equipped with Venus software v3 or higher.
 - QuantStudio® 5 Real-Time PCR System (Applied Biosystems A28139), 96-well, with QuantStudio® Design & Analysis Desktop Software version 1.5.1

Consumables

- a. Deep-well plates (DWP) (Thomas Scientific 1162C08)
- b. Elution microplate (Thomas Scientific 1144B80)
- c. 96-well PCR Plate (DN Biotech 5371009)
- d. Optical Adhesive Film (Applied Biosystems 4311971), to use in PCR instrument
- e. Adhesive film (Thomas Scientific 6980A10), to store RNA plate
- f. Small reagent tubes (Sarstedt 72.703)
- g. 1 mL tips (Hamilton 235940)

- h. 300 µL tips (Hamilton 235938)

Reagents

- a. 100% Ethanol, Molecular Biology Grade (Sigma E7023 or equivalent)

2. Components Required But Not Included with the AMPIPROBE® SARS-CoV-2 Test System for manual extraction and PCR set up (to be supplied by the user)

Instruments and Equipment

- a. Plate magnet (e.g. Alpaqua Enhanced Universal Magnet Plate, Alpaqua # A000400), for use with manual RNA extraction method
- b. Heater shaker (e.g. Bull Dog # 1808-0506), for use with manual RNA extraction method
- c. QuantStudio® 5 Real-Time PCR System (Applied Biosystems A28139), 96-well, with QuantStudio® Design & Analysis Desktop Software version 1.5.1

Consumables

- a. Deep-well plates (DWP) (Thomas Scientific 1162C08)
- b. 96-well PCR Plate (DN Biotech 5371009)
- c. Optical Adhesive Film (Applied Biosystems 4311971), to use in PCR instrument

Reagents

- a. 100% Ethanol, Molecular Biology Grade (Sigma E7023)

3. Components Required But Not Included with the AMPIPROBE® SARS-CoV-2 Assay and Controls Kit for automated extraction using the QIASymphony® SP Instrument and QIASymphony® DSP Virus/Pathogen Midi Kit (to be supplied by the user)

Instruments and Equipment

- a. QIASymphony® SP (QIAGEN # 9001297) with software version 4.0.2, for alternate automated extraction method
- b. QuantStudio® 5 Real-Time PCR System (Applied Biosystems A28139), 96-well, with QuantStudio® Design & Analysis Desktop Software version 1.5.1

Consumables & Reagents

- a. 100% Ethanol, Molecular Biology Grade (Sigma E7023)
- b. QIASymphony® DSP Virus/Pathogen Midi Kit (QIAGEN # 937055)

WARNINGS AND PRECAUTIONS

1. The assay is for *in vitro* diagnostic (IVD) use under the FDA Emergency Use Authorization Only.
2. For prescription use only.
3. The AMPIPROBE® SARS-CoV-2 Test System has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories; laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
4. The AMPIPROBE® SARS-CoV-2 Test System has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
5. The emergency use of the AMPIPROBE® SARS-CoV-2 Test System 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
6. 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.
7. The AMPIPROBE® SARS-CoV-2 Test System should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled under a laminar airflow hood or biological safety cabinet.
8. Kit components should be stored at the right temperatures as indicated on the labels. Care should be taken to limit the number of freeze-thaw cycles where applicable.
9. Always check the expiration date prior to use of reagents. DO NOT use expired reagents.
10. When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
11. Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures. Refer to 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>

12. Handle all samples and controls as if they are capable of transmitting infectious agents. Positive results are indicative of the presence of SARS-CoV-2 RNA.
13. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
14. Contamination may occur if carryover of samples is not adequately controlled during sample pool preparation, handling, and processing.
15. Testing of pooled specimens may impact the detection capability of the AMPIPROBE® SARS-CoV-2 Test System and impact sensitivity.
16. Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
17. Some of the AMPIXTRACT™ SARS-CoV-2 Extraction kit components contain hazardous substances. The Lysis Reagent, which contains sodium perchlorate, and ethanol, and Proteinase can be harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Consult the appropriate material safety data sheets (MSDS) for more information.
18. If liquid containing the buffers is spilt, clean with suitable laboratory detergent and water. Specifically, the AMPIXTRACT™ Lysis Reagent contains sodium perchlorate, a corrosive. Spills of this reagent should be avoided. If this reagent is spilt, clean up the instrument or surface with 70% ethanol.
19. If liquid containing potentially infectious agents is spilt, clean the area first with laboratory detergent and water, and then with 10% (v/v) sodium hypochlorite followed by 70% ethanol.
20. Practice aseptic technique when handling reagents to avoid introduction of contaminants that might interfere with assay interpretation.
21. The use of screw-cap tubes and barrier pipette tips is strongly encouraged to prevent samples and reagents from becoming aerosolized which might lead to contamination.
22. Avoid exposure of reagents to UV light (used for decontamination) or continuous exposure to light, especially the fluorogenic reagents. It may cause accelerated aging of the reagents and buffers.
23. Observe good laboratory practices. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. Any biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

QUALITY CONTROL

CONTROLS

The following controls provided in the AMPIPROBE® SARS-CoV-2 Control Kit (ENZ-GEN218-0004) must be included during PCR setup to accurately interpret patient results:

- One Positive Control (PC) that contains SARS-CoV-2 targets N1 and N2, and human RNase P target.
- One Negative Control (NC) that contains human RNase P target.
- One No Template Control (NTC) that do not contain any targets.

Note: Internal Control (IC), which is human RNase P, is present in human specimens. The AMPIPROBE® SARS-CoV-2 Test System utilizes a sequence from the human RNase P gene as the IC target. The presence of human RNase P should be detected in every patient specimen, and in Extraction Controls (EC), and Positive and Negative Controls (PC and NC). The internal control verifies that nucleic acid is present in the sample and is used for every sample processed. This is also used as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

Positive Controls (PC). The PC is the *in vitro* transcribed and purified viral SARS-CoV-2 RNA targets N1 and N2 and is needed to verify that the assay run is performing as intended. It is used in every RT-PCR assay plate starting at master mix addition, and 12 µL of PC would provide 108 copies in the PCR reaction and is equivalent to 1544 copies/ml of starting material. The positive control also includes an RNase P target and provides 2160 copies in PCR and will result as “Positive” for that marker.

Negative Control (NC). The NC is needed to verify that the assay run is performing as intended and is used on every assay plate starting at the master mix addition with no SARS-CoV-2 RNA target, but *within vitro* transcribed RNase P target at 2160 copies in PCR and will result as “Negative” for SARS-CoV-2.

No Template Control (NTC). The NTC is needed to eliminate the possibility of sample contamination on the assay run and is used once for each batch of extracted specimens and all subsequent steps (i.e., RT-PCR assay plate).

In addition to the above, an **Extraction Control (EC)** can also be included (optional). The Extraction Control (EC) to use is a previously confirmed negative patient sample that is taken through every extraction procedure. The presence of human RNase P should be detected in the extraction control. It serves as a negative control to monitor for any cross-contamination that occurs during the extraction process. Also, it validates successful RNA extraction and validation of extraction reagents.

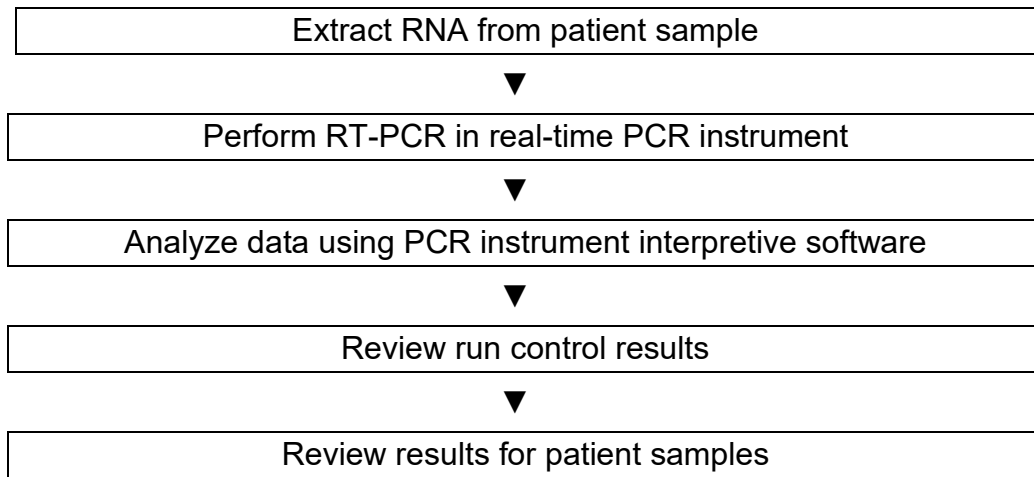
CORRECTIVE ACTION FOR INVALID RESULTS

1. If any control (PC, NC, or NTC) result is invalid, the PCR run is invalid and no patient results can be reported.
2. Report any QC failure to the supervisor immediately before taking corrective actions.
3. Corrective action, including reviewing sample processing workflow, decontamination of bench and/or workstations, repeat testing of QC and samples and consultation with kit and instrument manufacturers, will be taken and documented as needed. In general, if the controls are out of their expected range, all of the specimens and controls from that run must be processed beginning from the sample preparation step.

SPECIMEN COLLECTION / TRANSPORT / STORAGE

- For sample collection, handling and storage, refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19: <https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html>
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs should not be used and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 3 mL of viral transport media. A minimum of 1mL transport media is required to complete testing of pooled samples.
- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2–8°C and ship on ice packs
- Specimens can be stored at 2–8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

WORKFLOW



The workflow starts with the extraction of the RNA from upper respiratory specimens. RNA is isolated and purified from the specimen using the AMPIXTRACT™ SARS-CoV-2 Extraction Kit (Enzo ENZ-GEN216-0960) or QIASymphony® DSP Virus/Pathogen Midi Kit (QIAGEN # 937055). RNA isolation can be performed manually with the AMPIXTRACT™ SARS-CoV-2 Extraction Kit or via an automated process using the Enzo's GENFLEX® platform with the AMPIXTRACT™ SARS-CoV-2 Extraction Kit or the QIAGEN QIASymphony® SP Instrument with the QIASymphony® DSP Virus/Pathogen Midi Kit. For more information about using the GENFLEX® platform or the QIAGEN QIASymphony® SP Instrument, see sections **RNA EXTRACTION and PREPARATIONS of RT-PCR REACTIONS (Automated Method Using the Enzo GENFLEX® Platform)** and **RNA EXTRACTION (Automated Method Using the QIAGEN QIASymphony® SP)**. The AMPIXTRACT™ SARS-CoV-2 Extraction kit utilizes magnetic-particle technology for isolation and purification of nucleic acids from upper respiratory specimens. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities.

The purified RNA is reversed transcribed into cDNA and amplified using the AMPIPROBE® SARS-CoV-2 Assay kit (Enzo ENZ-GEN215-0096) in the Applied Biosystems QuantStudio® 5 Real-Time PCR Instrument (AB # A28569).

The data are analyzed, then interpreted by the QuantStudio® Design & Analysis Desktop Software version 1.5.1.

REAGENTS AND SAMPLE PREPARATION

EXTRACTION REAGENTS PREPARATION

1. **AMPIXTRACT™ Wash Reagent Working Solution**

Add 200 mL of 100% ethanol to each bottle containing the Wash Reagent solution. Store at room temperature until use.

2. **AMPIXTRACT™ Lysis Reagent Working Solution**

Add 40 mL of 100% ethanol to each bottle containing the Lysis Reagent solution. Mix thoroughly before use. Store at room temperature until use.

3. **AMPIXTRACT™ Stabilizer**

Place the vial(s) of Stabilizer on the GENFLEX® platform after the reagent is completely thawed.

SAMPLE PREPARATION

1. Bring the patient samples to room temperature. Prepare the sample collection tubes in the BSL2 cabinet. For upper respiratory swab samples, carefully uncap the sample tubes, and while holding the cap above the vial, carefully remove the swabs from the patient samples using a long forceps and discard the swabs into a biohazard waste container that contains 10% bleach solution. Alternatively, the patient samples can be removed to clean secondary vials and the samples can be processed in these vials as long as the vials have the patient information or barcodes for identification.

For sample pooling, samples must be collected in a minimum of 1mL transport media. Combine and mix 200ul each from up to five individual samples into a single tube for a final volume of 1mL. The decision to pool samples should be based on the positivity rate at each location. Please see Attachment 1 – Specimen Pooling Implementation and Monitoring _for further information regarding pooling.

2. Proceed to RNA extraction and process the samples according to one of the following options including at least one positive control and one negative control in the extraction run.

RNA EXTRACTION AND PREPARATION OF RT-PCR REACTIONS (Automated Method Using the Enzo GENFLEX® Platform)

The following procedures are for the automated RNA Extraction and RT-PCR reaction using Enzo's GENFLEX® platform (ENZ-GEN-FLX). The GENFLEX® platform includes a liquid handling workstation (Hamilton Microlab STAR™) and a RT-PCR instrument (Applied Biosystems QuantStudio® 5 Real-Time PCR Instrument).

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Thoroughly clean all work surfaces with fresh 10% bleach followed by fresh 70% alcohol before and after each use.
3. The GENFLEX® platform should be checked prior to daily runs. Documentation of these procedures is logged in the individual instruments' maintenance folder. We recommend following the maintenance instructions given in the user manual to reduce the risk of contamination.
4. It is recommended to power on the GENFLEX® platform, along with the external Inheco heating unit, before sample and reagent preparation is started so that the initialization process is complete before the instrument is needed.
5. Before starting a run, ensure that you have sufficient reagents for the batches you are planning to run.
6. Using extraction kits in combination with amplification systems that use extraction control require inclusion of this control into the purification procedure to monitor the efficiency of sample preparation and downstream assay.
7. Since small amounts of liquid are lost during transfer and contact with the magnetic particles, the initial volume of elution solution must be larger than the selected volume to ensure that the final eluate is of the correct volume.
8. Ribonucleases (RNases) are very stable and active enzymes generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plastic ware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.
9. PCR should be set up immediately after extraction of RNA. For short term or long-term storage, we recommend storage at -80°C avoiding repeated freeze-thaw cycles.

EXTRACTION PROCEDURE

Sample Preparation for Extraction

1. Place the Extraction Control (EC), a previously confirmed negative patient sample, in the sample carrier position 1.
2. After the swabs have been removed from the patient sample vials, place the specimen tubes with at least 1mL of an individual patient specimen, or 1mL of pooled sample (mix 200ul each from up to five individual samples into a single tube for a final volume of 1mL) starting at position **2** of the sample carrier. Load all the sample tubes onto additional sample carriers, if necessary.
3. Once the samples including the control are placed in the carrier with lids removed, bring them carefully from the BSL2 cabinet to the GENFLEX® platform for loading procedure.

Preparing the Reagents for the Run

1. Prepare **Complete Lysis/Binding Reagent** for n samples according to the following table:

Component	Volume (1 sample)*	Volume (N n samples + 10)*
Lysis Buffer	900 μ L	(900 x N) μ L
Magnetic Beads	20 μ L	(20 x N) μ L
Proteinase K	2 μ L	(2 x N) μ L
PK dilution Buffer	54 μ L	(54 x N) μ L
Carrier RNA	10 μ L	(10 x N) μ L
Stabilizer	14 μ L	(14 x N) μ L
Total volume per well	1000 μL	

* Include 10% overage when making master mix for multiple wells

2. Vortex to thoroughly mix the **Complete Lysis/Binding Reagent**.
3. Unwrap the 200 mL and 20 mL troughs before use. Refer to **Table 1** below.

TABLE 1. Working Solutions and Trough Size	
Working Solutions to be Loaded	Trough size
AMPIXTRACT™ Wash Reagent	200 mL
AMPIXTRACT™ Complete Lysis/Binding Reagent	200 mL
AMPIXTRACT™ Elution Buffer	20 mL

Transfer the reagents from the bottles provided in the kit to the troughs following the prompts as they appear on the instrument's screen

Extraction Procedure on the GENFLEX® Platform

Note: The sample input volume for extraction is 350 μL and the nucleic acid eluate volume is 60 μL .

1. Power on the instrument, if not already done so. The power switch (green switch) is located at the bottom-left corner of the instrument.
2. Turn on the Inheco heating unit after the GENFLEX® platform is turned on. The power switch for the heating unit is located at the back of the unit.
3. Click on the “Method Manager” icon on the desktop.
4. Select “Universal RAPID Extraction” icon from the list that appears on the screen.
5. Click on the green “Run” icon next to the COVID-19 method to initiate the run.
6. Once the instrument has finished its startup protocol and initialization, a user prompt box will be displayed within an additional window named “Rerack Tips”. Select the appropriate checkbox to either rerack tips or discard them in the waste. Click “continue” and in the next window, choose “96” well format for the PCR setup.
 - If you selected to rerack tips, a prompt will appear later during setup to load two empty tip racks with empty wafers at positions 16-21 and 22-27.
7. In the next screen, select “Sample Transfer Tube (32 position carrier)” as the sample type and input the number of samples loaded onto the instrument, including the extraction control.
8. Follow instructions that appear next on the screen. The screen will display a checklist of reagents to be loaded onto the deck of the GENFLEX® platform as shown in **Figure 1**. This checklist also serves as instructions on the transfer of each reagents to troughs and placement of the troughs in specific locations along given tracks.

Figure 1. Deck layout diagram for reagent input as shown in the GENFLEX® Venus Software.



9. **Figure 1** details the reagent loading positions highlighted in orange. Load the individual troughs with the appropriate volumes of the extraction kit reagents as indicated in **Table 2** below for 93 samples.

TABLE 2. Volume of Extraction Kit Reagents to be Loaded into individual Troughs			
Loading Positions	Working Solutions to be Loaded	Volume Loaded	Track #s
1	AMPIXTRACT™ Wash Reagent Working Solution	187 mL	13-14
2	AMPIXTRACT™ Complete Lysis/Binding Reagent	103 mL	13-14
5	AMPIXTRACT™ Elution Buffer	9 mL	15

10. Once the reagents are loaded as indicated, and the list is checked, click “continue”.

11. Load the sample tubes and consumables including the racks of filter tips, deep well plate and the empty PCR elution plate as indicated in **Figure 2**. Follow the Deck layout as indicated in the diagram and **Table 3** to identify the carriers and positions to complete the loading process.

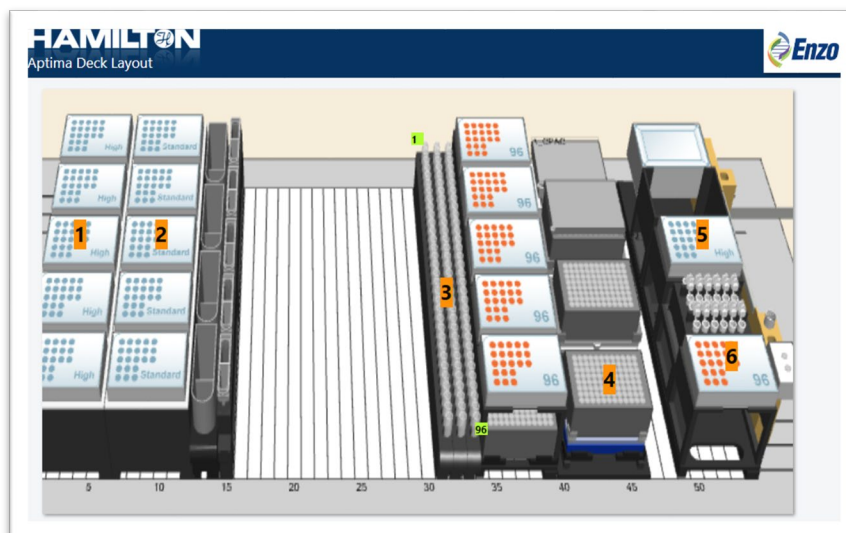


Figure 2. Deck layout diagram for consumables as shown in the GENFLEX® Venus Software.

TABLE 3. Consumables to be Loaded			
Loading Positions	Consumables	Cat. No.	Track #s
1	1 mL filter tips	Hamilton 235940	1-6
2	300 µL filter tips	Hamilton 235938	7-12
3	Sample tubes in carriers starting in track 31 loaded onto the loading tray	BioMerieux 69285	31-34
4	2 mL Deepwell Extraction plate "ON MAGNET"	Thomas Scientific 1162C08	40-46
5	Full rack of 1 mL filter tips in Tip module	Hamilton 235940	49-54
6	Empty RNA elution plate	DN Biotech 5371009	49-54

12. Place the sample tubes with the extraction control and the specimens in the carrier and slide the carrier in through the tracks onto the loading platform. The barcode reading autoloader will pull the sample carrier into the deck of the instrument once the run commences. Until then, the sample carriers should remain on the loading platform. Place the Deepwell Extraction plate "on magnet" as indicated in the table above and an empty PCR plate that serves as an elution plate on the PCR module as indicated on the deck layout and in the table above.
13. Click "Continue" after the consumable loading process is complete. The "continue" tab will only appear after the selection of the items in the

checklist.

14. The next screen that appears will display the minimum number of 1 mL tips needed for the extraction for the inputted number of samples.
15. Click “Continue”. The next window will display the layout of the 1 ml tips ondeck. See **Figure 3**. Load the necessary number of tips until the plastic sleeve that holds each 96 tips snap into the tip carrier of the deck. Make sure that the number of tips loaded on deck at least meets or exceeds the number of tips necessary for the extraction.

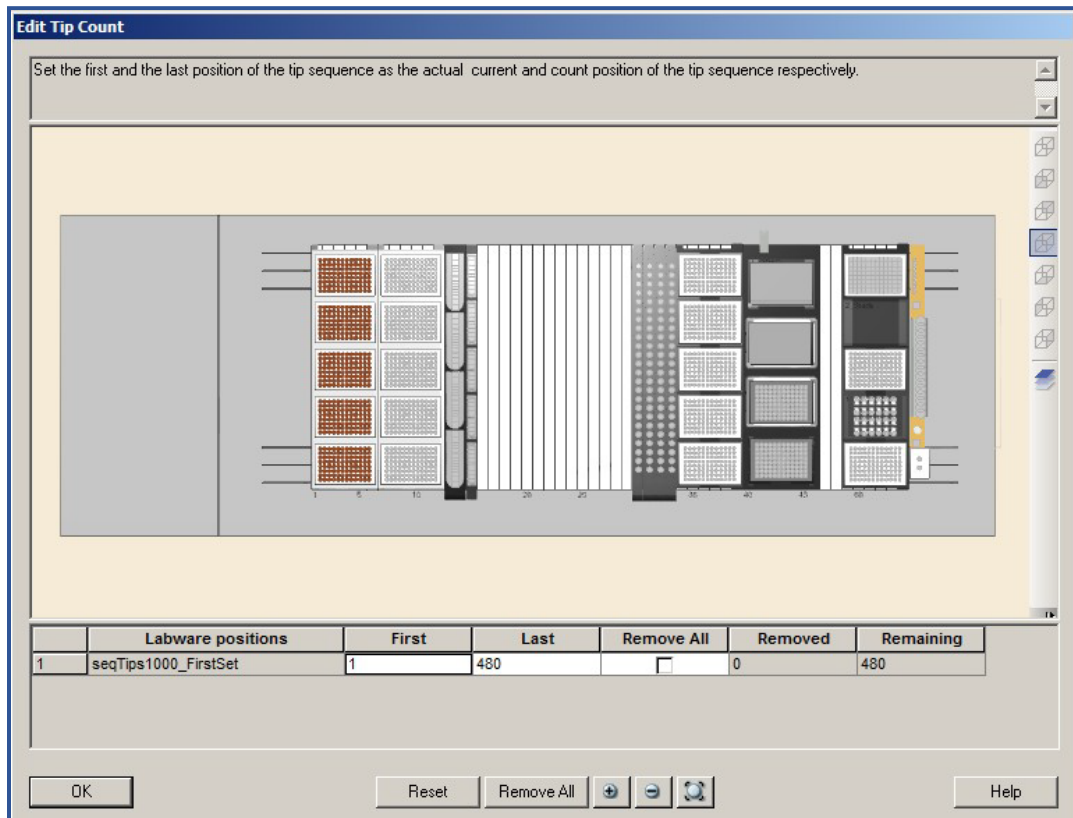


Figure 3. Location of loaded tips necessary for the run in GENFLEX® Venus Software

16. In the interactive display, input actual location of 1 mL tips as loaded on the deck by clicking or dragging the tip positions. When selected, the loaded positions will be highlighted in brown.
17. Ensure that the physical arrangement of the 1 mL tips that were just loaded on the deck is identical to the arrangement of 1 mL tips highlighted in brown. If more tips needed to be added, zoom in on the display, and then select either individual tip locations, columns or the entire 96-well format by clicking or dragging the tip positions. Click “OK”

after the positions of the tip sequence are updated in brown in the display matching the actual deck arrangement of the tips.

18. The following screens will display the number of 300 µL tips needed for the run as well the input window that requires the location of the tips to be updated will be displayed. Update the number of tips on the window displayed and then click “OK”.
19. The extraction will start and several windows showing timers for incubation steps will appear during the run. No input from the user is necessary until the run finishes.
20. Once the run is completed, a window stating “AMPIPROBE extraction is now complete” will be displayed.
21. Click “continue” to close the “Run Control” window of the software.
22. The samples are now ready for downstream applications. Keep the RNA elution plate sealed with the adhesive film at 2-8°C if PCR is being carried out within 2-3 hours, or at -80°C for later use.

RT-PCR Master Mix (MMX) Preparation

Note: *The MMX preparation should be performed inside a PCR hood, and this hood must reside outside of the post-amplification area (preferably in the pre-amp room). All the reagents used in the MMX preparation are from the AMPIPROBE® SARS-CoV-2 Assay kit (ENZ-GEN215-0096 or ENZ-GEN215-0960) and all controls are from the AMPIPROBE® SARS-CoV-2 Controls kit (ENZ-GEN218-0004).*

1. Remove all of the RT-PCR reagents from AMPIPROBE® SARS-CoV-2 Assay kit box and all PCR Controls from the AMPIPROBE® SARS-CoV-2 Controls kit box. Thaw the reagents at room temperature while protected from light for 10-15 minutes, except the AMPIPROBE® RT-PCR Enzyme Mix. The RT-PCR Enzyme Mix from the kit should always remain on ice or in the -20°C freezer until needed for addition. All the other reagents, after being thawed, should be placed in an ice bucket or cool rack for holding during preparation.
2. The amount of MMX reagents required for the procedure can be calculated using the following table (**Table 4**).

TABLE 4. RT PCR Master Mix (MMX)		
AMPIPROBE® MMX Reagents	Volume per Sample or Control	Calculation for the Aliquot Procedure (n* + 26)
RT-PCR Buffer	10 µL	10 x (n + 26) µL
SARS-Cov-2 Primer/Probe Mix	2 µL	2 x (n + 26) µL

RT-PCR Enzyme Mix	1 μ L	1 x (n + 26) μ L
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*n is the number of samples prepared, which includes the 3 AMPIPROBE® SARS-CoV-2 Controls and 1 Extraction Control (EC).

- Once the amount of each reagent has been determined, use a 1.5 mL tube to prepare the MMX in. Vortex each tube in the kits (except for the Enzyme Mix) for three seconds and then briefly centrifuge them to remove any excess from the caps.
- Add the MMX reagents in the order given in table above for the PCR setup. Instead of vortexing the RT-PCR Enzyme Mix, simply flick the tube gently three times and briefly centrifuge to remove excess from the cap, and then add the required volume to complete the MMX. Once the MMX is prepared, invert the tube ten times, briefly centrifuge, and then place on ice or at 2-8°C, protected from direct light until it is needed for the PCR setup.

Note: Prepared MMX may be kept for up to 20 minutes before loading and starting the Applied Biosystems QuantStudio® 5 Real-Time PCR Instrument.

- Proceed to setting up for MMX aliquoting and sample RNA template addition by the GENFLEX® platform.

GENFLEX® Platform Setup for PCR Reactions

Note: The GENFLEX® platform uses the Venus Software, Version 3 or higher.

- If PCR is being setup immediately following the extraction, do not power off the GENFLEX® platform or close the Method Manager window on the desktop. If the instrument is powered off, turn the power switch on. The power switch (green switch) is located at the bottom-left corner of the instrument.
- Double click on the “Method Manager” icon on the desktop of the computer. Click on “Run” next to COVID-19 icon, and select the “PCR” from the User Input Run Selection window.
- In the next window displayed, select “96” for the 96-well format for the PCR, and click “continue”. Select the Sample Barcode file that has the sample information from ‘C:\Outputfiles\SampleBarcodes’. Select the Sample Barcode file with the correct time stamp and click Continue. A dialog box will appear to select if controls will be used in PCR. Select “Yes” and click “Continue”.
- In the next window displayed, type in the number of samples for PCR setup as shown as on the third line of **Figure 4**. This would be similar to the format followed in the extraction. For an entire plate, 92 patient specimens can be processed along with one extraction control, which would be 93 samples. The number of PCR plates to prepare would be 1, the Master Mix volume would be 13 μ L and

the DNA template column, which is RNA, would be 12 μ L out of the 60 μ L total eluate from the extraction. In the next window, select the samples from the plate map. Note that the number of samples selected on the map must match the number of samples entered in the previous window. If the number of samples selected on map does not match the number of samples entered, an error message will prompt you to re-select samples on the map.

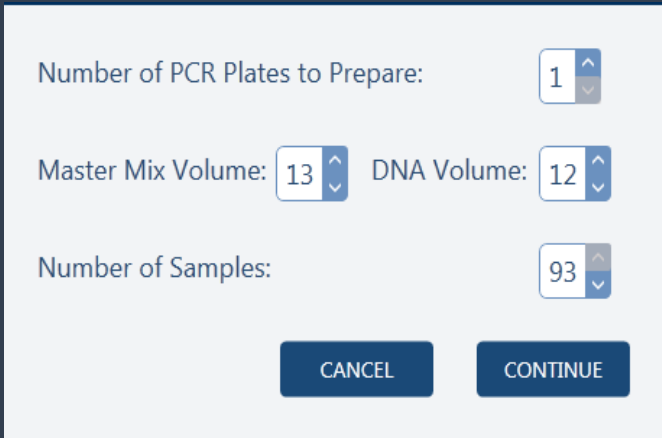
The image shows a software input window for PCR setup. It contains three rows of input fields, each with a label and a numeric spinner control. The first row is labeled 'Number of PCR Plates to Prepare:' with a spinner set to '1'. The second row has two spinners: 'Master Mix Volume:' set to '13' and 'DNA Volume:' set to '12'. The third row is labeled 'Number of Samples:' with a spinner set to '93'. At the bottom of the window are two buttons: 'CANCEL' and 'CONTINUE'.

Figure 4. Input window displaying the number of samples for PCR.

5. In the next window displayed, choose the plate to which you want to add the controls (only one plate required for COVID assay, so the standard protocol is to have Plate 1 selected). Click continue and in the next window displayed, select multi-dispensing options. The standard protocol for COVID method is to have master mix multi-dispensed, and the RNA single-dispensed. The Deck Layout diagram showing locations of filter tips, PCR plate, RNA plate and the vials with standards and Master Mixes to be loaded is displayed next as shown in **Figure 5**. Load the filter tips, followed by the plates and the standards as shown in the diagram. After checking the list, click continue. A window to load and confirm 300 μ L tip locations will appear similar to the diagram as shown in **Figure 3**. The multi-dispensing and the PCR setup will start and a window displaying “AMPIPROBE PCR setup complete” indicates the completion of the setup. Seal the plate with the adhesive film.

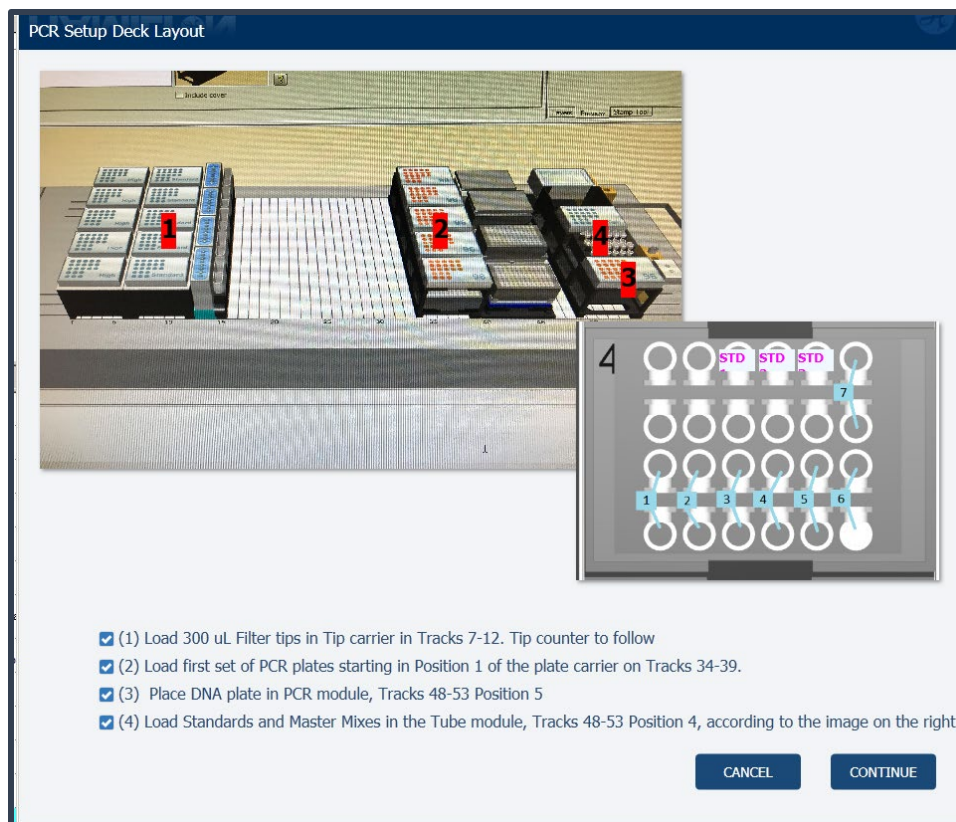


Figure 5. Diagram to load the tips, plates and the vials with locations specified.

6. Perform the RT-PCR using the procedure as described in **RT-PCR USING THE APPLIED BIOSYSTEMS™ QUANTSTUDIO® 5 REAL-TIME PCR INSTRUMENT** section. .

RNA EXTRACTION (Automated Method Using the QIAGEN QIAasymphony® SP)

The following automated RNA extraction procedure is performed on the QIAasymphony® SP instrument (QIAGEN # 9001297) and using QIAasymphony® DSP Virus/Pathogen Midi Kit (QIAGEN # 937055). For more information on the QIAGEN instrument and the purification kit, refer to the manufacturer's instruction manuals.

Before You Begin

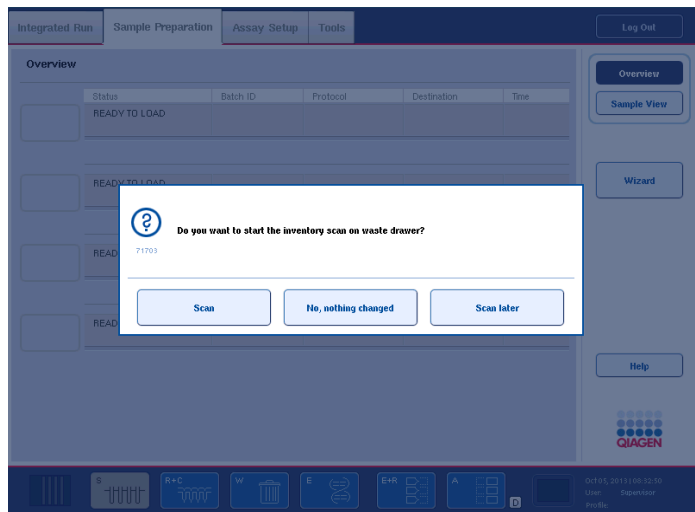
1. Equilibrate upper respiratory specimens to room temperature for 30 minutes. Transfer 0.65 mL of each specimen into a labelled 2 mL sample tube. The sample input volume is 0.5 mL.
2. Thaw a vial(s) of Carrier RNA and dilute in 1350 µL AVE Buffer. Store unused diluted Carrier RNA solution in -80°C freezer.
3. An internal control will be required for this extraction method. To prepare the

control, mix 5 µL of pre-diluted Carrier RNA (from step 2) with 106 µL AVE Buffer. This Carrier RNA solution will serve as the internal control for the isolation run.

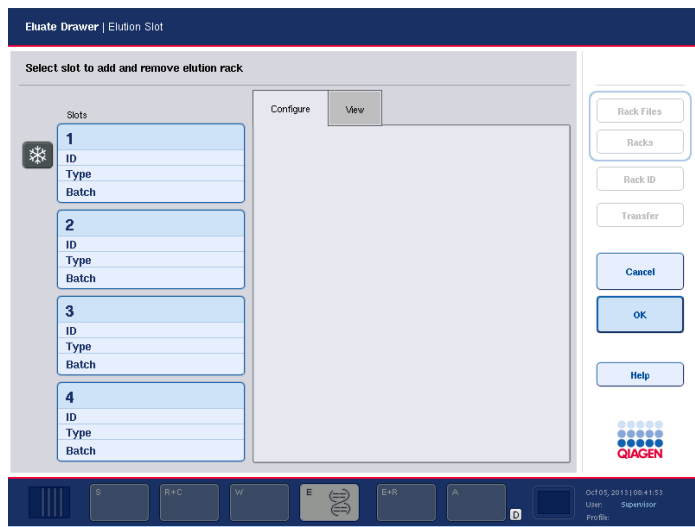
4. Before using a reagent cartridge (RC) in QIAAsymphony® DSP Virus/Pathogen midi kit for the first time, check that Buffers QSL2 and QSB1 do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.
5. Make sure that the piercing lid (PL) is placed on the reagent cartridge (RC) and the lid of the magnetic-particle trough has been removed or, if using a partially used reagent cartridge (RC), make sure the Reuse Seal Strips have been removed.
6. Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
7. Before loading the reagent cartridge (RC), remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the enzyme has been equilibrated to room temperature (15–25°C). Make sure that the piercing lid (PL) is placed on the reagent cartridge (RC) or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
8. If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAAsymphony® SP.

RNA Extraction Using QIAAsymphony® SP

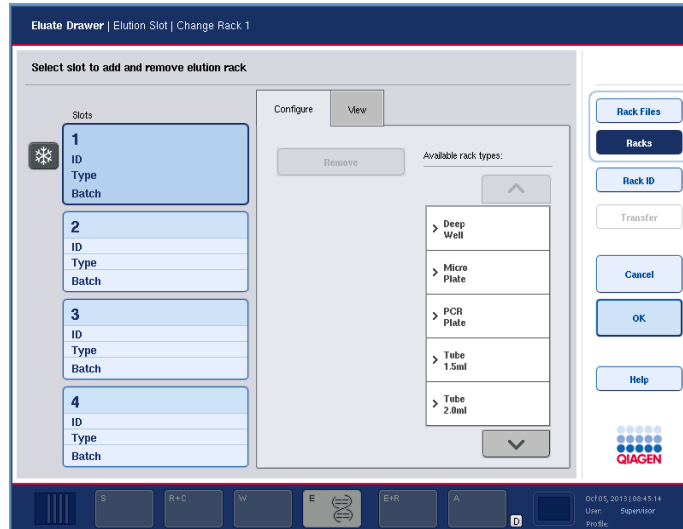
1. Make sure all drawers and the hood of QIAAsymphony® SP machine are closed.
2. Push the power button to switch on the QIAAsymphony® SP. Wait until the “Sample Preparation” screen appears, and the initialization procedure has finished.
3. Click “Log in” on top right panel and log in with user account.
4. Ensure the “Waste” drawer is prepared properly by ensuring that there is adequate space for liquid and solid waste to be added. Close the draw and perform an inventory scan by select “Scan” and perform an inventory scan of the “Waste” drawer, including the tip chute and liquid waste as shown below. Replace the tip disposal bag if necessary.



5. Load the required elution rack into the “Eluate” drawer. Use only “Elution slot 1” with the corresponding cooling adapter. When using a 96-well EMTR plate, make sure that the plate is in the correct orientation, and the bottom of the plate box is removed, as incorrect placement may cause sample mix-up in downstream analysis. Label the required amount of tubes, load into the elution rack with cap open as shown below, and close the “Eluate” drawer.

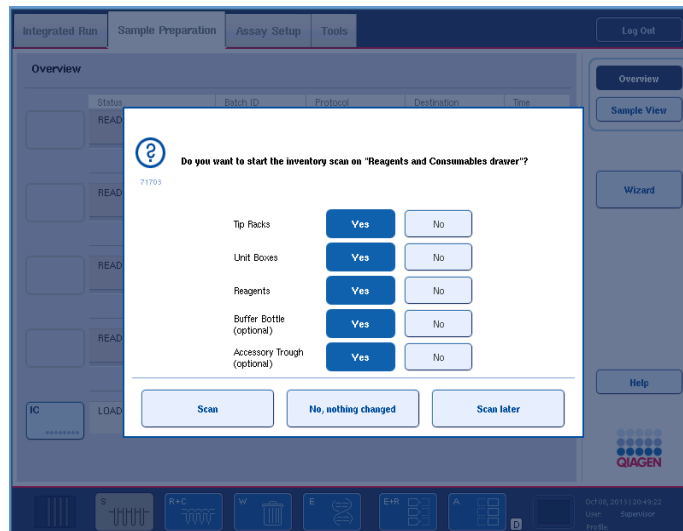


Choose position 1 for the cold rack as shown below.



Choose correct rack type (EMTR plate is under Deep Well and 2 mL tubes are under Tube 2.0 mL) and input rack ID, press “OK” as shown above and let machine perform the scan.

6. Add required Sample Prep Cartridges, Rod Covers, Tips, and Reagents into the corresponding positions in “Reagents and Consumables” drawer. Close the drawer and perform an inventory scan by clicking “Scan” as shown below.

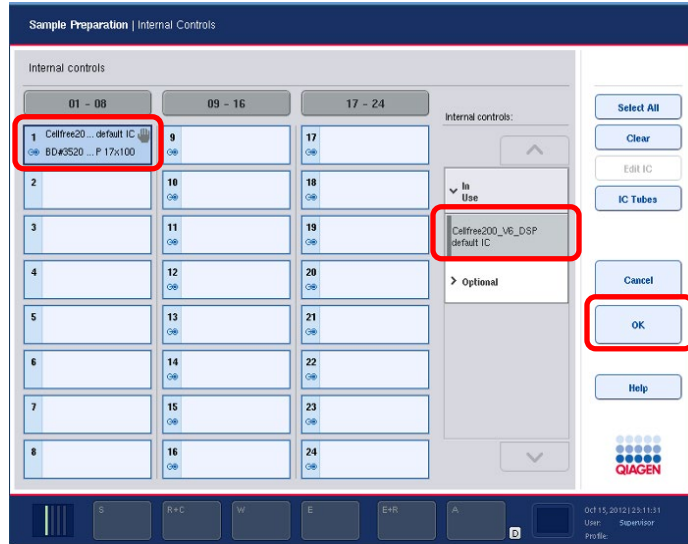


7. Place the tube(s) containing the Carrier RNA (CARRIER)–Buffer AVE (AVE) mixture as well as an additional tube of the Carrier RNA in Buffer AVE in the place of internal control into the tube carrier and load into slot **A** of the “Sample” drawer. This step can be performed while the machine is scanning for reagent and consumables.

Select “IC” to get into “Internal Controls” screen, select the required tube and

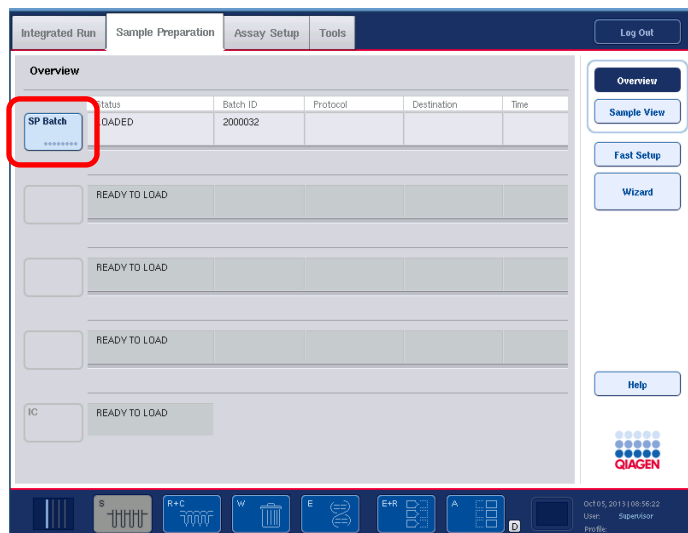
define the tube type and choose “CellFree_500_v4_DSP” as the protocol, then click ‘OK’ as shown below.

Note: manufacturer’s extraction instructions have been modified for use, we recommend using “Cellfree500_V4_DSP” protocol instead of “Complex 200”, “Complex 400”, or “Complex 800”, which are the manufacturer’s recommended protocols for respiratory samples.

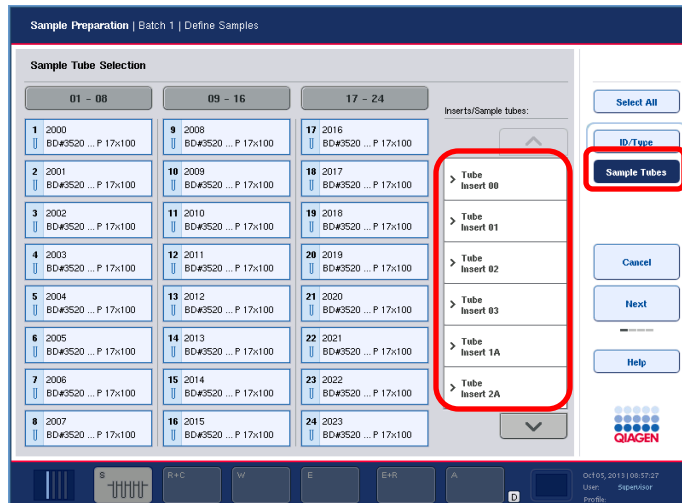


- Place the samples into the sample carrier with tube 3B adapter and load them into the “Sample” drawer slot 1-4.

On the Sample Preparation tab, select the batch to be processed as shown below.



Check that the correct sample tube plastic-ware and barcodes were recognized by the barcode scanner as shown below. The 2.0 mL tube is under “Tube Insert 3B” tab. If there are no barcodes on the samples, the ID’s will need to be entered manually, and the samples will be highlighted yellow.



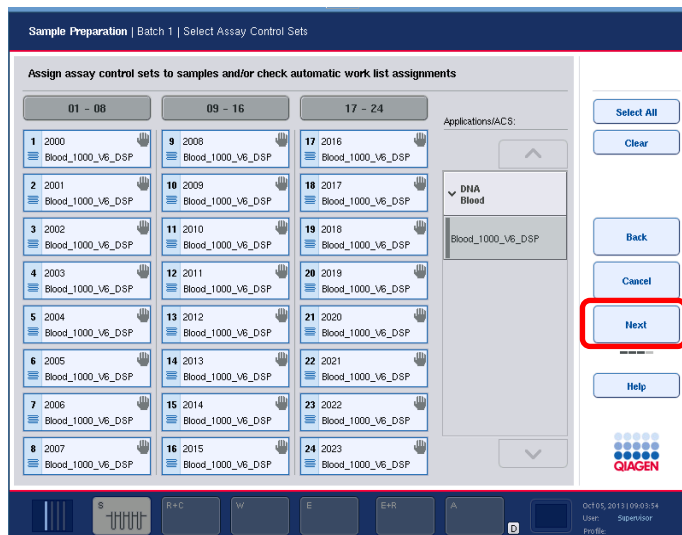
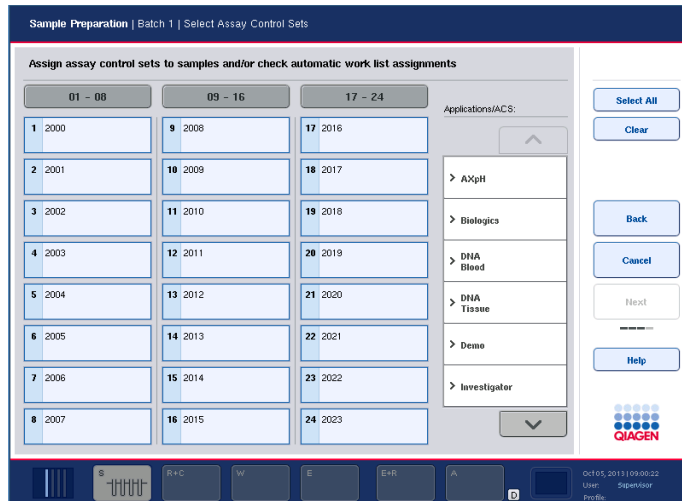
To enter ID's manually, select 'ID Type', then 'Sample ID', ensuring that a sample is selected (highlighted blue), on the next screen enter the sample ID and select 'OK'. Once a sample ID has been entered it can also be defined as a 'Sample' as shown below.



Click 'OK' to go to the next step.

9. Select the required samples and assign the relevant extraction protocol to those samples as shown below. Choose "Cellfree500_V4_DSP" protocol from "Virus" in Applications menu.

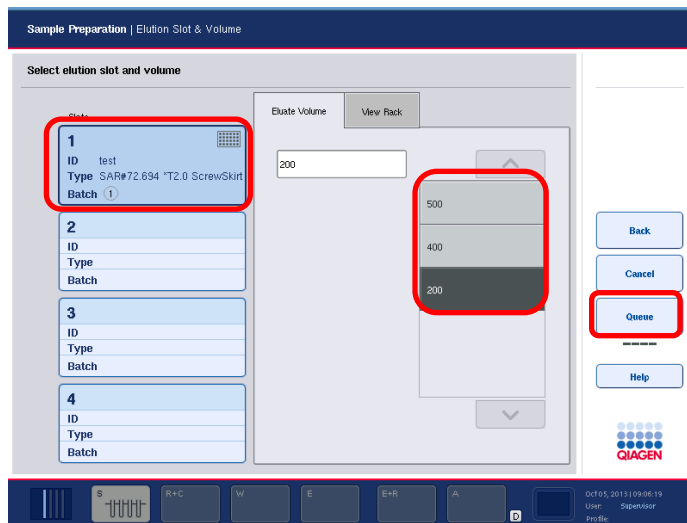
Note: manufacturer's extraction instructions have been modified for use, we recommend using "Cellfree500_V4_DSP" protocol instead of "Complex 200", "Complex 400", or "Complex 800", which are the manufacturer's recommended protocols for respiratory samples.



10. Once a method has been assigned the 'Next' button becomes available and can be selected.

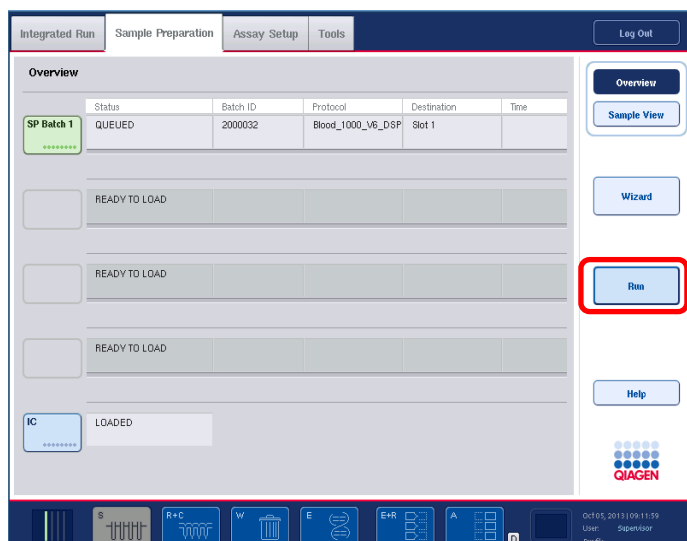
On the next screen, you need to select the elution position (this should be the same position that you loaded earlier on the Eluate draw) as shown below.

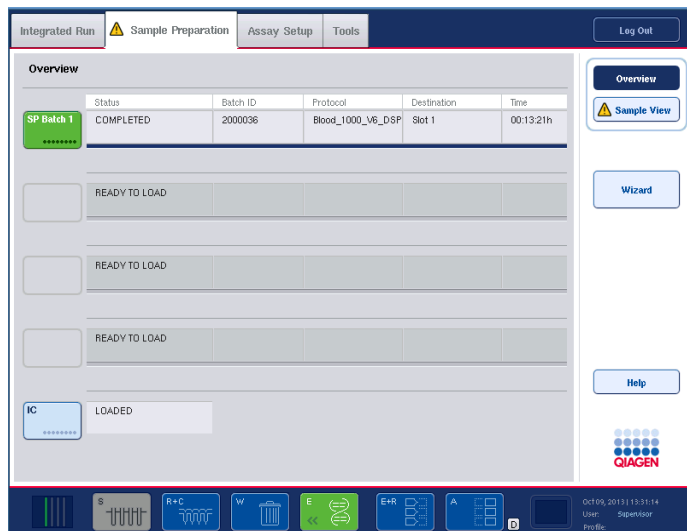
Select the block and then select the desired elution volume as 60 μ L from the options provided.



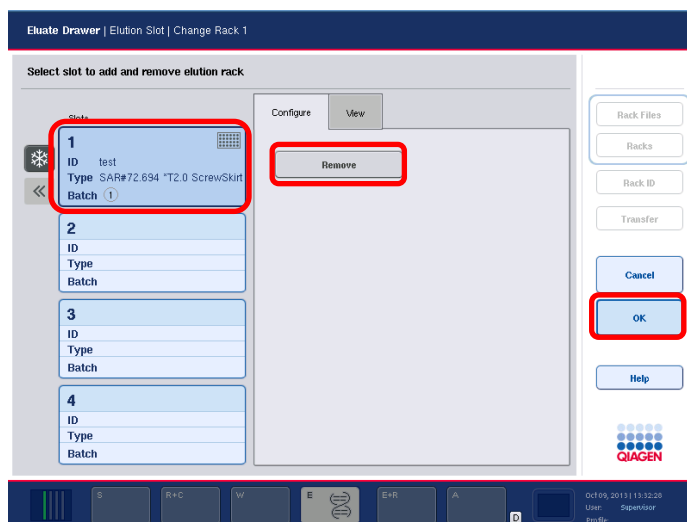
Then select 'Queue' to queue the run on the system. This will return you to the overview screen on the Sample Preparation Tab.

- Once the entire inventory scan has completed. Press the "Run" button to start the purification procedure. All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED" as shown below.

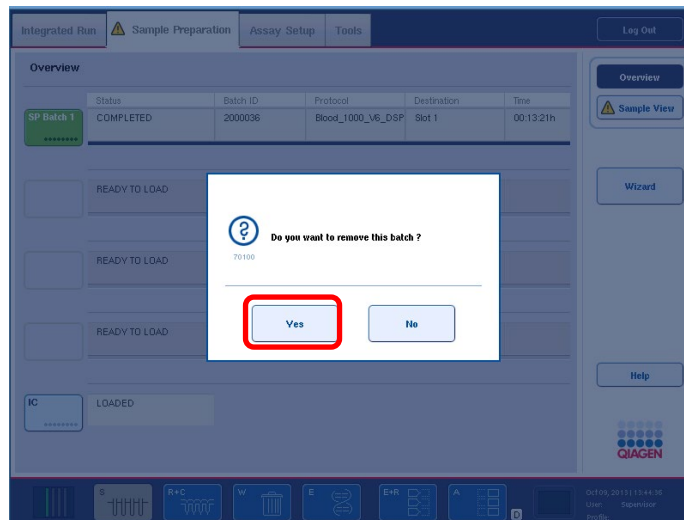
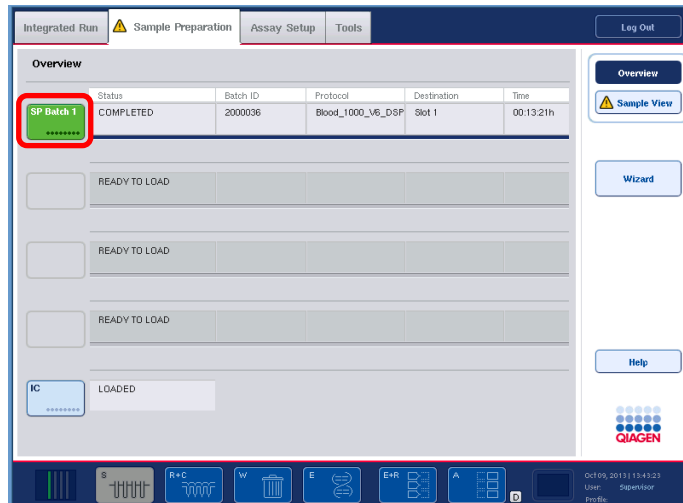




- Once the run is complete, open the Eluate drawer and remove the block, cap the samples and store/use as appropriate. On the screen, select the position and select 'Remove'. Close the draw and press 'OK'.



- On the overview screen, select the batch to be removed. Select 'Yes' to remove the batch. Unload the samples from the carrier draw and remove the reagents. Then the machine will be ready for the next run.



14. For long-term storage, store purified nucleic acids at -80°C or else proceed to setting up for PCR.
15. Immediately seal the partially used reagents with the provided Reuse Seal Strips and close tubes containing Proteinase K with screw caps after the end of the protocol run to avoid evaporation.
16. Discard used sample tubes, plates, and waste according to your local safety regulations.
17. Clean the QIAasymphony[®] SP by wiping with 70% ethanol.
18. Close the instrument drawers, and log off the user and switch off the QIAasymphony[®] SP.
19. Set up the RT-PCR reaction using the manual procedure as described in the following section, **RT-PCR REACTION PREPARATION (Manual Method)**.

RNA EXTRACTION AND PREPARATION OF RT-PCR REACTIONS (Manual Method)

RNA EXTRACTION (Manual Method)

Manual extraction can be performed from a sample input volume of 350 μL using the AMPIXTRACT™ SARS-CoV-2 Extraction kit, ENZ-GEN216.

Before You Begin

1. Determine the number of required nucleic acid purifications (n) based on the number of samples to be processed and controls per plate.
2. Thaw **Stabilizer and Carrier RNA** reagents and place on ice.
3. Bring all samples to room temperature.
4. Prepare the AMPIXTRACT™ Wash Reagent and Lysis Reagent working solutions as described in **REAGENTS AND SAMPLE PREPARATION** section.
5. Prepare **Complete Lysis/Binding Reagent for n samples** according to the following table:

Component	Volume (1 sample)*	Volume (n samples)*
Lysis Buffer	900 μL	(900 x n) μL
Magnetic Beads	20 μL	(20 x n) μL
Proteinase K	2 μL	(2 x n) μL
PK dilution Buffer	54 μL	(54 x n) μL
Carrier RNA	10 μL	(10 x n) μL
Stabilizer	14 μL	(14 x n) μL
Total volume per well	1000 μL	

* Include 10% overage when making master mix for multiple wells

6. Vortex to thoroughly mix the **Complete Lysis/Binding Reagent**.

Process the Samples

Note: *If any magnetic beads are accidentally drawn into the pipette tip during processing the liquid should be ejected back into its well. Place the plate back on the plate magnet and carefully re-aspirate the liquid after the beads have settled.*

1. Add 350 μ L of samples to the wells of a Deep Well Plate (DWP).
2. Lyse the samples by adding 1000 μ L Complete Lysis/Binding Reagent to each sample well. Incubate the plate for 25 minutes on the heater shaker set at 56°C and 1100 rpm for efficient lysing.
3. After incubation, place the plate on a plate magnet for a 96-well plate (e.g. Alpaqua # A000400) for 3 minutes. Aspirate the supernatant carefully without dislodging the bead pellet and discard the supernatant.
4. Wash and dry the beads.
 - 4.1 Remove the plate from the plate magnet, then add 950 μ L of the Wash Buffer (pre-mixed with ethanol) to each sample well. Incubate the plate for 2 minutes on the heater shaker set at 56°C and 1200 rpm.
 - 4.2 Place the plate on the plate magnet for 1 minute, aspirate the supernatant and discard.
 - 4.3 Remove the plate from the plate magnet and repeat the wash step by adding 950 μ L Wash Buffer (pre-mixed with ethanol), and then incubate for 2 minutes on the heater shaker set at 56°C and 1200 rpm.
 - 4.4 Place the plate on the magnetic stand for 1 minute, and then aspirate and discard the supernatant. Completely remove washings to facilitate efficient drying of the beads in the next step.
 - 4.5 Allow the beads to dry by placing the plate for 10 minutes on the heater shaker set at 65°C and 500 rpm. Make sure the beads are completely dry before proceeding to the elution step (step 6). To ensure efficient elution, beads should be completely dry.
5. Elute the RNA.
 - 5.1 Add 60 μ L of Elution Buffer to each well. Incubate the plate for 10 minutes at 56°C while shaking at 1400 rpm for elution of the nucleic acid.
 - 5.2 Place the plate on the plate magnet for 1 minute. Remove and save the supernatant or eluate.
 - 5.3 Store the eluate, which contains the extracted RNAs, at -80°C for later use. Seal the plate immediately to prevent evaporation before transferring to -80°C storage.

RT-PCR REACTION PREPARATION (Manual Method)

The procedure described below is for using the extracted sample RNA using an original sample input volume of 350 µL.

Before You Begin

1. To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area.
2. Do not use the same pipette for controls and samples. Always use aerosol barrier pipette tips.
3. The following controls provided in the AMPIPROBE® SARS-CoV-2 Control Kit, ENZ-GEN218-0004, must be included for each run to accurately interpret patient results:
 - One Positive Control
 - One Negative Control
 - One No-Template Control

In addition to the above, an **Extraction Control (EC)** can also be included. The Extraction Control (EC) to use is a previously confirmed negative patient sample that is taken through every extraction procedure. For additional information, refer to the **Quality Control** section.

Preparation of the Master Mix (MMX)

The Master Mix (MMX) is prepared using the reagents provided in the AMPIPROBE® SARS-CoV-2 Assay kit, ENZ-GEN215-0096.

1. The amount of MMX reagents required for the procedure can be calculated using the following table.

AMPIPROBE® MMX Reagents	Volume per Sample	Calculation for the Aliquot Procedure ($n^* + 3$)
RT-PCR Buffer	10 µL	10 x ($n + 3$) µL
SARS-Cov-2 Primer/Probe Mix	2 µL	2 x ($n + 3$) µL
RT-PCR Enzyme Mix	1 µL	1 x ($n + 3$) µL

* n is the number of samples prepared, which includes the 3 AMPIPROBE® SARS-CoV-2 Controls and 1 Extraction Control (EC).

2. Once the amount of each reagent has been determined, use a 1.5 mL tube to prepare the MMX in. Vortex each reagent from the kit (except for the RT-PCR Enzyme Mix) for three seconds and then briefly centrifuge to collect liquid at the bottom of the tubes.
3. Add the MMX reagents in the order given in table above for the PCR setup.

Note: DO NOT vortex the RT-PCR Enzyme Mix. Simply flick the tube gently three times, and then briefly centrifuge to collect liquid at the bottom of the tube.

Then add the required volume of the RT-PCR Enzyme Mix to complete the MMX. Once the MMX is prepared, invert the tube ten times, briefly centrifuge, and then place on ice or at 2-8°C, protected from direct light, until it is needed for the PCR setup.

Note: Prepared MMX may be kept for up to 20 minutes on ice before loading and starting the Real-time PCR instrument.

RT-PCR USING THE APPLIED BIOSYSTEMS™ QUANTSTUDIO® 5 REAL-TIME PCR INSTRUMENT

The procedure described below is for setting up and running RT-PCR using Applied Biosystems QuantStudio® 5 Real Time PCR instrument. For more information about the QuantStudio® 5 Real Time PCR instrument, refer to the instrument's manual.



Figure 6. Applied Biosystems QuantStudio® 5

Make sure the Applied Biosystems QuantStudio® 5 (shown in **Figure 6**) is powered on first, followed by the instrument's computer. The power switch for the instrument is located on the back of the instrument. Once both the instrument and computer have finished their start-ups, open the software “QuantStudio® Design and Analysis” on the desktop. On the touch screen of the PCR instrument, go to “File” → “New Experiment” → “From template” → “COVID template 2”. Then proceed as follows:

1. From the File menu, click “Import Plate Setup” and “Plate Import” file. Enter the file name with any relevant notes about the run (i.e. load name and date) in the “Experiment Properties” tab; and then click “Next”.

Verify that the temperature and cycling profile matches the settings given in **Table 5** below. Click “Next” and in the “Advanced Setup” tab, make sure the number of

samples selected under the “Sample” tab matches the number of samples being processed. Make sure FAM, HEX, and Cy5 are shown and selected as “Targets”. The green channel, which is FAM, corresponds to SARS-CoV-2 N1 target, the yellow channel, which is HEX, corresponds to the RNase P target, and the red channel, which is Cy5, corresponds to the SARS-CoV-2 N2 target.

TABLE 5. PCR Cycling and Temperature Conditions for the SARS CoV 2 Assay				
Parameter	Reverse Transcription	Enzyme Activation	Amplification/ Detection	
Cycles	1	1	40	
Segment	1	1	1	2
Target [°C]	50	95	95	58
Hold [mm:ss]	30:00	02:00	00:03	00:30

2. Load the PCR plate containing the RNA and Master Mix into the PCR instrument. In order to load the plate, touch the eject instrument drawer icon (1) located on the top right as shown in **Figure 7**, and place the PCR plate with the reaction mixture into the drawer. Click “Start Run” in the Run tab.



Figure 7. Home Screen of Applied Biosystems QuantStudio® 5

3. Click on “Start Run” to begin the run.
4. Once the run is finished with the procedure, the raw data needs to be analyzed, it will be done to establish the result determinations for each sample.

DATA ANALYSIS

The data analysis described below and the results interpretation in the following section are using Applied Biosystems QuantStudio® 5 Real-Time PCR instrument and QuantStudio® Design & Analysis Desktop Software version 1.5.1. For additional information, refer to the manufacturer's manual.

C(t) Determination

1. The amplification and detection performed on Applied Biosystems QuantStudio® 5 will be analyzed in the software to obtain the threshold crossing, C(t), for each channel.
2. For the PCR run, the green channel corresponds to SARS-CoV-2 N1 target, the yellow channel corresponds to RNase P (RP), and the red channel corresponds to the SARS-CoV-2 N2 target.

INTERPRETATION OF RESULTS AND REPORTING

The expected control sample results and interpretation are listed below. All controls must yield valid results for a test run to be valid. Only after it is determined that all controls are valid patient samples can be interpreted. If any of the controls below fail that run is invalid and patient samples cannot be interpreted. Patient samples have to be re-run after the root cause is identified and eliminated. See **Table 6** below for control sample interpretation.

TABLE 6. Control Sample Interpretation				
Control	SARS-CoV-2 N1 C(t)	SARS-CoV-2 N2 C(t)	RNase P C(t)	Interpretation
Positive Control	< 38.0	< 38	<35	Valid result
Extraction Control	Negative, C(t) not detected	Negative, C(t) not detected	<35	Valid result
No Template Control (NTC)	Negative, C(t) not detected	Negative, C(t) not detected	Negative, C(t) not detected	Valid result
Negative Control	Negative, C(t) not detected	Negative, C(t) not detected	< 35	Valid result

Patient Sample Interpretation:

Only if a test run is valid can the results for individual patient samples be interpreted and reported. N1 and N2 are considered positive (+) in the Table 7 below when the Ct < 38. RNase P is considered positive (+) when the Ct < 35

TABLE 7. Patient Sample Interpretation				
N1 Target	N2 Target	RNase P	Result Interpretation	Action
+	+	+/-	Detected	Report results to sender and appropriate public health authorities
If only one target is positive		+/-	Inconclusive	Repeat extraction and rRT-PCR once. If the repeated result remains the same, it is reported as Inconclusive and recommend recollection if clinically indicated.
-	-	+	Not Detected	Report results to sender.
-	-	-	Invalid	Repeat extraction and rRT-PCR once. If the repeated result remains invalid, consider collecting a new specimen from the patients.

Examination and Interpretation of Pooled Patient Specimen Results

Only if a test run is valid can the results for a patient sample pool be interpreted and reported. For a patient sample pool, N1 and N2 are considered positive (+) in Table 8 below when the Ct < 38. RNase P is considered positive (+) when the Ct < 35

Negative – Negative results from pooled sample testing should not be treated as definitive. If the patient’s clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive - Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Inconclusive - Specimens with an inconclusive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing

Invalid - Specimens with an invalid pool result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled

specimens may be appropriate depending on laboratory workflow and required result reporting time.

TABLE 8. Pooled sample interpretation				
N1 Target	N2 Target	RNase P	Result interpretation	Action
+	+	+/-	Pooled Detected – DO NOT REPORT	Individually test each sample from the pool
Either N1 or N2 positive		+/-	Pooled Inconclusive-DO NOT REPORT	Individually test each sample from the pool
-	-	+	Not Detected	Report results to sender
-	-	-	Invalid	Individually test each sample from the pool

LIMITATIONS

- The use of this assay as an *In vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- The AMPIPROBE® SARS-CoV-2 Test System can be used with the upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) listed in the Intended Use statement. Other specimen types have not been evaluated and should not be tested with this assay.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- Laboratories are required to report all results to the appropriate public health authorities.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may affect the test performance.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been validated.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the test target region, SARS-CoV-2 RNA may not be detected or may be detected less predictably. Inhibitors or other types of

interference may produce a false negative result.

- False-positive results may arise from cross contamination during specimen handling, preparation, nucleic acid extraction, PCR assay set-up or product handling.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic, immunosuppressant drugs or cold medications have not been evaluated.
- Nasopharyngeal wash/aspirate and nasal aspirates specimen types should not be pooled.
- Sample pooling has only been validated using nasopharyngeal swab specimens.
- Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

CONDITIONS OF AUTHORIZATION

The AMPIPROBE® SARS-CoV-2 Test System's Letter of Authorization, User Manual, and Labeling are available on FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

To assist clinical laboratories using the AMPIPROBE® SARS-CoV-2 Test System, the relevant Conditions of Authorization are listed below.

- Authorized laboratories^[b] using the AMPIPROBE® SARS-CoV-2 Test System must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the AMPIPROBE® SARS-CoV-2 Test System must use your test as outlined in the Instructions for Use only. 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.
- Authorized laboratories that receive the AMPIPROBE® SARS-CoV-2 Test System must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the AMPIPROBE® SARS-CoV-2 Test System must have a process in place for reporting test results to healthcare providers and relevant public health authorities.
- Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Enzo Life Sciences (techserv-usa@enzolifesciences.com; 1-800-942-0430) if they become aware of any

suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the AMPIPROBE® SARS-CoV-2 Test System.

- All laboratory personnel using the AMPIPROBE® SARS-CoV-2 Test System must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your test in accordance with the authorized labeling.
- Authorized laboratories using specimen pooling strategies when testing patient specimens with the authorized test must include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that “Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.
- Authorized laboratories implementing pooling strategies for testing patient specimens must use the “Specimen Pooling Implementation and Monitoring Guidelines” provided in the authorized tests’ Instructions for Use/Package Insert to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.
- Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Strategies. For the first 12 months from the date of their creation, such records must be made available to FDA within 48 business hours for inspection upon request, and must be made available within a reasonable time after 12 months from the date of their creation.
- Enzo Life Sciences, its authorized distributor(s) and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

^(b) The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”

PERFORMANCE CHARACTERISTICS

1) Performance evaluation of automated extraction and PCR set up on the GENFLEX® platform using the AMPIPROBE® SARS-CoV-2 Test System

Limit of Detection, LoD (Analytical Sensitivity)

The LoD is defined from this study as the lowest concentration of SARS-CoV-2 that can be reliably detected at least 95% of the time. The preliminary LoD was determined testing triplicates of eight samples prepared by spiking the SeraCare AccuPlex™ SARS-CoV-2 Reference Material (Material Code 0505-0126, Batch #10480311, SARS-CoV-2 genomic RNA sequences ORF1a, RdRp, E gene, and N gene) at a concentration ranging from 10 to 4,230 copies/mL. The LoD was confirmed by testing 20 replicates at the preliminary LoD. The confirmatory sample was prepared by spiking the SeraCare material into nasopharyngeal swabs in VTM previously tested negative for SARS-CoV-2. In Table 9, the study results show that the LoD for the AMPIPROBE® SARS-CoV-2 Test System is 280 copies/mL

Table 9. Limit of Detection (LoD) of the AMPIPROBE® SARS-CoV-2 Test System for detection of SARS-CoV-2 N1 and N2 target

Replicate Group No.	SARS CoV 2 RNA copies/mL	No. of Samples Tested	No. of Samples Positive	Mean C(t)	Positivity (%)
N1	280	20	19	36.4	95
N2	280	20	20	35.1	100

2) Performance evaluation of AMPIEXTRACT™ SARS-CoV-2 Extraction kit using a manual extraction method, and AMPIPROBE® SARS-CoV-2 Assay and Controls kits for amplification and detection using Applied Biosystems QuantStudio® 5 Real-Time PCR instrument

Limit of Detection (LoD) side by side comparison (Manual vs Automated extraction on GENFLEX® extraction platform)

Samples were prepared by spiking SeraCare AccuPlex™ SARS-CoV-2 Reference Material into nasopharyngeal swabs in VTM previously tested negative for SARS-CoV-2. A total of five concentrations of SeraCare material were tested in this study in parallel by the AMPIPROBE® SARS-CoV-2 Test System using the manual extraction and using the automated extraction with the GENFLEX® platform. The study results show that the LoD for the AMPIPROBE® SARS-CoV-2 Test System with manual extraction is 93 copies/mL and is comparable to results obtained when using the GENFLEX® platform.

3) Performance evaluation of QIAGEN QIASymphony® DSP Virus/Pathogen Midi Kit using automated extraction on QIAGEN QIASymphony® SP, and AMPIPROBE® SARS-CoV-2 Assay and Control kits for amplification and detection using Applied Biosystems QuantStudio® 5 Real-Time PCR instrument

Limit of Detection (LoD) side by side comparison (Automated on QIAGEN QIASymphony® SP vs Manual with AMPIXTRACT™ SARS-CoV-2 Extraction kit)

Samples were prepared by spiking SeraCare AccuPlex™ SARS-CoV-2 Reference Material into nasopharyngeal swabs in VTM previously tested negative for SARS-CoV-2. A total of four concentrations of SeraCare material were tested in this study in parallel by the AMPIPROBE® SARS-CoV-2 assay using the automated extraction with the QIASymphony® SP and using manual extraction with the AMPIXTRACT™ SARS-CoV-2 Extraction kit. The study results show that the LoD for the AMPIPROBE® SARS-CoV-2 Assay on samples isolated using the QIASymphony® SP instrument with the QIASymphony® DSP Virus/Pathogen Midi Kit is 280 copies/mL and is comparable to results obtained when using the AMPIXTRACT™ SARS-CoV-2 Extraction kit. The isolation protocol used on the QIASymphony® SP instrument was “Cellfree500_V4_DSP” starting with 500 µl of sample, and eluting with 60 µl.

Inclusivity (Analytical Sensitivity)

The AMPIPROBE® SARS-CoV-2 Test System uses the same primer and probe sequences as the FDA authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The CDC provided updated inclusivity analyses on June 20, 2020 (CDC-006-00019, Revision: 05) that assessed 31,623 SARS-Coronavirus-2 sequences. A single nucleotide mismatch was identified at the third position of the N1 probe in 2% of sequences. The frequency of all other mismatches against the remaining primers and N2 probe was less than 1%.

The likelihood of one or two mismatches resulting in a significant loss of reactivity is low because the mismatches are not in a critical region of the primers, and the T_m of the primers and probes are such that they can tolerate one or two mismatches. In addition, the dual target design of the test mitigates the failure to detect SARS-CoV2 due to mismatches in any one target.

Cross-reactivity (Analytical Specificity)

To determine the cross-reactivity of the AMPIPROBE® SARS-CoV-2 Test System, wet testing was done with results shown in **Table 10** and *in silico* analysis was done with results shown in **Table 11**.

Wet Testing Results

For wet cross-reactivity testing, 20 individual patient samples that tested positive for a variety of non-SARS-CoV-2 viral respiratory infections on the BioFire Film Array

Respiratory Panel 2 at Enzo Clinical Labs, Farmingdale, NY (CLIA Certificate No. 33D0159701), were tested by the AMPIXTRACT™ SARS CoV-2 Extraction and AMPIPROBE® SARS-CoV-2 Assay Kits. None of the 20 samples had SARS-CoV-2 targets detected by the AMPIPROBE® SARS-CoV-2 Test System.

Table 10. Cross-reactivity wet testing using the AMPIPROBE® SARS-CoV-2 Extraction and AMPIPROBE® SARS-CoV-2 Assay Kits performed on clinical sample positive for other viral respiratory infection (non-SARS-CoV-2)

No.	Patient Sample	SARS CoV 2 N1	SARS CoV 2 N2	Result
1	Coronavirus NL63	NOT DETECTED	NOT DETECTED	NOT DETECTED
2	Coronavirus HKU1	NOT DETECTED	NOT DETECTED	NOT DETECTED
3	Coronavirus HKU1	NOT DETECTED	NOT DETECTED	NOT DETECTED
4	Coronavirus HKU1	NOT DETECTED	NOT DETECTED	NOT DETECTED
5	Coronavirus NL63	NOT DETECTED	NOT DETECTED	NOT DETECTED
6	Coronavirus NL63	NOT DETECTED	NOT DETECTED	NOT DETECTED
7	Coronavirus NL63	NOT DETECTED	NOT DETECTED	NOT DETECTED
8	Coronavirus HKU1	NOT DETECTED	NOT DETECTED	NOT DETECTED
9	Coronavirus HKU1	NOT DETECTED	NOT DETECTED	NOT DETECTED
10	Coronavirus NL63	NOT DETECTED	NOT DETECTED	NOT DETECTED
11	Coronavirus OC43, Human Rhinovirus / Enterovirus, Influenza B	NOT DETECTED	NOT DETECTED	NOT DETECTED
12	Coronavirus HKU1, Coronavirus NL63	NOT DETECTED	NOT DETECTED	NOT DETECTED
13	Coronavirus 229E	NOT DETECTED	NOT DETECTED	NOT DETECTED
14	Coronavirus HKU1, Influenza A H1-2009	NOT DETECTED	NOT DETECTED	NOT DETECTED
15	Coronavirus HKU1, Human Rhinovirus / Enterovirus, Respiratory Syncytial Virus	NOT DETECTED	NOT DETECTED	NOT DETECTED
16	Coronavirus OC43	NOT DETECTED	NOT DETECTED	NOT DETECTED
17	Coronavirus HKU1, Coronavirus NL63, Respiratory Syncytial Virus	NOT DETECTED	NOT DETECTED	NOT DETECTED
18	Coronavirus HKU1, Human Metapneumovirus	NOT DETECTED	NOT DETECTED	NOT DETECTED
19	Coronavirus 229E, Human Rhinovirus /Enterovirus	NOT DETECTED	NOT DETECTED	NOT DETECTED
20	Coronavirus HKU1, Human metapneumovirus	NOT DETECTED	NOT DETECTED	NOT DETECTED

In Silico Analysis Results:

Homology with other organisms likely to be found in upper respiratory samples was determined for each of the primers and probes using the NCBI Blastn megablast search against each of the target organisms, using the non-redundant Nucleotide collection on May 7, 2020. The search parameters were automatically adjusted for short input sequences and the expected threshold was 10. The match and mismatch scores were 2 and -3 respectively. The penalty to create and extend a gap in the alignment was 5 and 2 respectively.

In silico cross-reactivity testing with the % homology of the AMPIPROBE® SARS-CoV-2 Test System primers and probes is provided in Table 11 for various organisms that are reasonably likely to be encountered in the respiratory clinical specimens.

With the exception of SARS-CoV (SARS1), all other organisms did not show greater than 80% homology to the AMPIPROBE® SARS-CoV-2 Test System primers and probes and it can be reasonably concluded that the primers and probes will not amplify and detect the virus, bacterial or yeast sequences analyzed.

With regards to SARS-CoV (SARS1), N1-R primer, N1-P probe and N2-F primer showed greater than 80% homology to the AMPIPROBE® SARS-CoV-2 Assay primers and probes. However, the CDC EUA assay (FDA submission number EUA200001) which shares the same primer and probe sequences as the AMPIPROBE® SARS-CoV-2 Test System primers and probes showed via wet testing with SARS-CoV isolate tested in triplicate for N1 and N2 that there was no cross reactivity. As a result, it can be reasonably concluded that the AMPIPROBE® SARS-CoV-2 Test System primers and probes will not amplify and detect SARS-CoV.

Table 11. *In silico* cross-reactivity testing with the % homology of the AMPIPROBE® SARS-CoV-2 Test System primers and probes

	GenBank Accession #	N1 F	N1 R	N1 P	N2 F	N2 R	N2 P
Human coronavirus 229E	KY073748	55%	46%	42%	55%	50%	43%
Adenovirus (e.g. C1 Ad. 71)	LC215437	55%	38%	42%	55%	50%	48%
Human Metapneumovirus (hMPV)	MK588636	55%	54%	42%	55%	61%	48%
Parainfluenza virus 1-4	MH006684	75%	38%	38%	50%	44%	43%
Influenza A	MK339871	80%	58%	58%	65%	67%	52%
Influenza B	MT243145	50%	46%	50%	55%	56%	52%
Enterovirus (e.g. EV68)	JX417823	65%	46%	50%	65%	67%	57%
Respiratory syncytial virus	MK947289	55%	38%	42%	45%	44%	39%
Rhinovirus	JX417823	65%	58%	50%	65%	44%	57%
<i>Chlamydia pneumoniae</i>	LN849022	60%	58%	50%	55%	44%	57%
<i>Haemophilus influenzae</i>	LR134171	65%	50%	58%	70%	67%	57%
<i>Legionella pneumophila</i>	NZ_LBAR01000014	70%	58%	67%	80%	50%	63%
<i>Mycobacterium tuberculosis</i>	NZ_PYQC01000231	75%	58%	58%	80%	54%	75%
<i>Streptococcus pneumoniae</i>	NZ_CLUU01000012	70%	79%	58%	75%	71%	58%
<i>Streptococcus pyogenes</i>	NZ_CAAINE010000001	70%	58%	58%	65%	67%	54%
<i>Bordetella pertussis</i>	NZ_CSNY01000285	65%	0%	0%	60%	72%	50%
<i>Mycoplasma pneumoniae</i>	NZ_CP010546	60%	50%	54%	55%	56%	46%
<i>Pneumocystis jirovecii</i> (PJP)	NW_017264783	65%	54%	54%	65%	61%	54%
<i>Candida albicans</i>	NC_032094	65%	54%	79%	70%	67%	54%
<i>Pseudomonas aeruginosa</i>	NZ_NRCA01000015	75%	63%	63%	80%	78%	67%
<i>Staphylococcus epidermis</i>	NZ_VDZY01000730	65%	54%	54%	70%	72%	54%
<i>Staphylococcus sp.</i>	NZ_MRYW01000001	75%	67%	67%	70%	54%	71%
Human coronavirus OC43	MN310478	50%	42%	42%	60%	44%	48%
Human coronavirus HKU1	MH940245	50%	42%	42%	50%	56%	35%
Human coronavirus NL63	LC488390	45%	42%	42%	50%	50%	48%
MERS	MT387202	50%	50%	50%	55%	61%	48%
SARS1	MK062184	40%	92%	92%	100%	72%	43%

Clinical Evaluation

Clinical performance with individually tested specimens

A clinical evaluation of the AMPIPROBE® SARS-CoV-2 Test System on the GENFLEX® platform was performed in a blinded study with 200 patient nasopharyngeal swab specimens with results shown in **Table 12**. Comparator results for clinical samples were obtained from FDA EUA tests.:

Table 12. Clinical performance of the AMPIPROBE® SARS-CoV-2 Test System in nasopharyngeal swab specimens

		FDA EUA Tests			Total
		Positive Patient Specimen	Inconclusive specimen	Negative Patient Specimen	
AMPIPROBE®	Positive Patient Specimen	51	0	0	51
	Inconclusive	1	0	3	4
	Negative Patient Specimen	1	0	144	145
	Total	53	0	147	

The positive and negative percent agreements between the AMPIPROBE® SARS-CoV-2 Test System and FDA EUA tests are:

Positive Percent Agreement = $51/53 = 96.2\%$, CI [87.3% - 99.0%]

Negative Percent Agreement = $144/147 = 98.0\%$, CI [94.2% - 99.3%]

Inconclusive Rate = $4/200 = 2.0\%$

Note: There was insufficient material to re-test samples with inconclusive results.

Clinical performance with pooling of 5 specimens

The clinical performance of the AMPIPROBE® SARS-CoV-2 Test System was evaluated with a pool size of 5 specimens. Testing included positive and negative nasopharyngeal (NP) specimen pools. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. 25 positive and 20 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of the assay and included 5 (20%) low positive specimens (defined as

within 3 Ct of the assay LoD). Both the pooled and individual specimens were evaluated with the AMPIPROBE® SARS-CoV-2 Test System. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result for each evaluated pool size, as shown in Table 13.

Table 13: Results of pooled vs. individual samples including positive percent agreement and negative percent agreement

		n	Pooled samples				% Agreement
			Positive	Inconclusive	Negative	Invalid	
Individual samples	Positive	25	24	1*	0	0	100% (25/25) 95% CI: 83.89% - 100%
	Negative	20	0	1**	19	0	95.0% (19/20) 95% CI: 76.39% - 99.11%

* Since constituents of any pool that is not negative is re-tested as an individual sample, the PPA will include pools with positive or inconclusive results

** Any pool that is not negative (ie. inconclusive, positive, or invalid) is re-tested as an individual sample.

Pooling validation *in silico* sensitivity

An *in silico* analysis was performed to assess the loss of sensitivity (Ct value shift) caused by pooling five samples into a single extraction. An estimate of the Ct value shift was calculated using data generated from twenty-five positive samples that were processed both individually and as a pool of five after combining with four negative samples. The Passing-Bablok regression was applied to these data where individual Ct values from positive samples form the X-axis and Ct values from corresponding pools are on the Y-axis. Figures 8 and 9 include the Passing-Bablok regression fit (solid red line) as well as a reference curve with a slope of 1 and a Y-intercept of 0 (dashed blue line).

Figure 8. Passing-Bablok regression analysis for individual versus pooled sample processing for N1

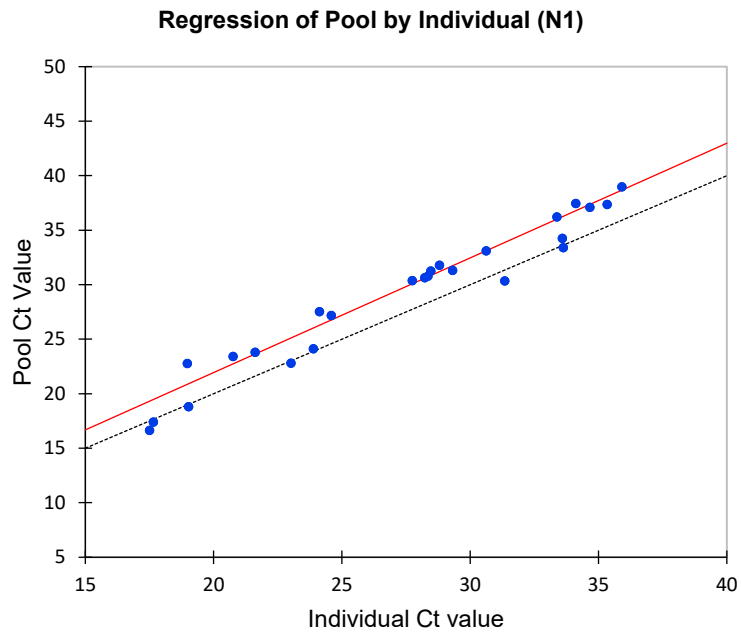
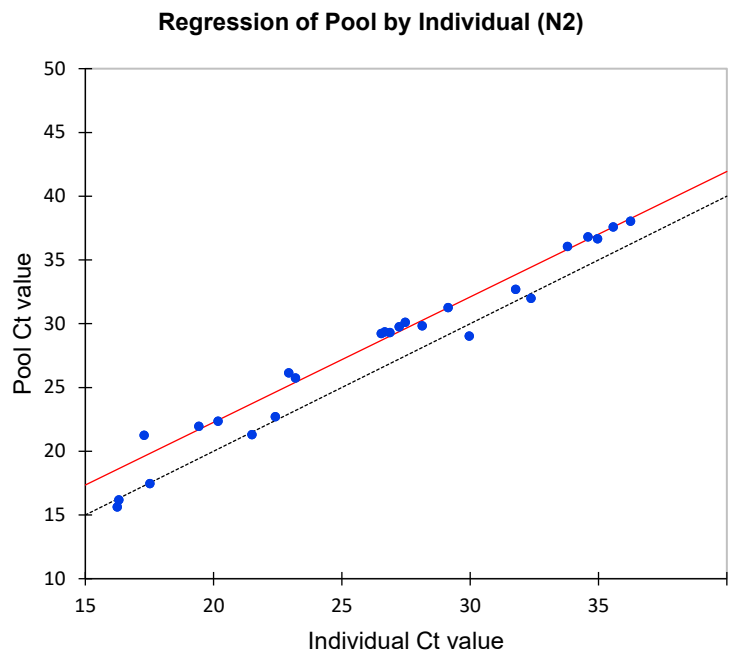


Figure 9. Passing-Bablok regression analysis for individual versus pooled sample processing for N2



trial

Table 14. Summary of Passing-Bablok regression analysis

Target	Intercept	Intercept (95% LCI)	Intercept (95% UCI)	Slope	Slope (95% LCI)	Slope (95% UCI)
N1	0.905	-3.604	3.208	1.052	0.972	1.191
N2	2.597	-1.404	4.303	0.983	0.925	1.108

LCI, lower confidence interval; UCI, upper confidence interval

Table 15. Ct value shift observed from sample pooling (n = 5)

Target	Regression equation [X^* , 38]	X^*	Δ Ct (regression)	Δ Ct (observed)
N1	$38 = 1.052X + 0.905$	35.27	2.73	1.73
N2	$38 = 0.983X + 2.597$	36.00	2.00	1.59

Using the regression analysis shown in Table 14 and a Ct value cutoff of 38 a shifted Ct value threshold can be used to estimate the number of weak positive samples that may be missed due to the pooling dilution effect (Table 15). The observed shift of the Ct value was also calculated by determining the mean difference between individual samples and their corresponding pools (Table 15).

***In-silico* sensitivity analysis of clinical sample data sets from 3 geographically diverse sites in the U.S.**

An *in silico* analysis was performed to assess the loss of sensitivity (Ct value shift) caused by pooling five samples into a single extraction. The Ct value shift was calculated using data generated from twenty-five positive samples that were processed both individually and as a pool of five after combining with four negative samples. The positive percent agreement was calculated for three geographically diverse US sites using the Ct value shift applied to the individually tested samples. The positive percent agreement was > 95% for each of the three sites (i.e., 95.7% for site 1 and 100% for sites 2 and 3).

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Nucleic acid extraction was performed as per our EUA-authorized method on the QIASymphony® SP instrument using the QIASymphony DSP/Virus/Pathogen Midi Kit. RT-PCR was performed using the AMPIPROBE® SARS-CoV-2 Assay on the Applied BioSystems™

QuantStudio® 5 Real Time PCR instrument. All runs included AMPIPROBE® SARS-CoV-2 Controls. Blinded sample testing was used to establish specificity and to confirm the LoD. The results are summarized in Table 16 below.

Table 16. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross- Reactivity
SARS-CoV-2	NP	3600 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

Attachment 1 – Sample Pooling Implementation and Monitoring (Laboratory Responsibilities)

Sample Pooling Implementation (Laboratory Monitoring Part A)

After the sample pooling using the candidate test has been authorized by the FDA, before a test developer validated sample pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate in the testing population and pooling testing efficiency (Table 1).

Table 1. Efficiency of pooling based on the positivity of SARS-CoV-2 RNA in individual samples (as an example)

P, percent of positive subjects in the tested population	$n_{\text{maxefficiency}}$ (n corresponding to the maximal efficiency)	Efficiency of n-sample pooling corresponding to $n_{\text{maxefficiency}}$ (a maximum increase in the number of tested patients when Dorfman n- pooling strategy used)
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

A.1 If Historical Data for Individual Specimens is Available

A.1.1 Positivity Rate of Individual Testing

- Estimate positivity rate ($P_{\text{individual}}$) in the laboratory based on individual sample testing. For this consider the 7-10 previous days and calculate the number of patients tested during those days. $P_{\text{individual}}$ is the number of positive results divided by the total number of tested patients during these 7-10 days.

A.1.2 Selection of test developer validated size of sample pools, n

- Use $P_{\text{individual}}$ and Table 1 to choose an appropriate validated pool size. Table 1 presents the pool size with the maximum efficiency for the validated pool sizes and positivity rates. If the positivity rate ($P_{\text{individual}}$) is in Table 1, choose n from Table 1 which corresponds to the maximum efficiency (F).
- If $P_{\text{individual}}$ in your laboratory does not correspond to the largest validated pool size in Table 1, the pool size with maximum efficiency for this positivity rate was not validated and you should choose the maximum n which was validated. For example, for the calculation of efficiency of 5-sample pooling, using formula $F=1/(1+1/5-(1-P)^5)$, when $P_{\text{individual}}$ is 1%, the efficiency F is 3.46 for n=5. It means that 1,000 tests can cover testing of 3,460 patients on average.
- If $P_{\text{individual}}$ is greater than 25%, then pooling patient samples is not efficient and should not be implemented.

A.2 If Historical Individual Data for Individual Specimens is Unavailable

If historical data from the previous 7-10 days is unavailable, the maximum pool size validated in the EUA and any smaller pool sizes can still be implemented, as the EUA test has been validated for the maximum pool size-specimen pooling. However, note that without $P_{\text{individual}}$, the laboratory may choose a pooling size that does not maximize pooling efficiency.

Sample Pooling Monitoring (Laboratory Monitoring Part B)

After implementing a n-sample pooling strategy, calculate the percent positivity rate (P_{pool}) based on n sample pooling strategy periodically using the data from pooled samples from the previous 7-10 days*.

B.1 If Historical Data for Individual Specimens is Available

If historical data for individual specimens is available, compare P_{pool} to $P_{\text{individual}}$ periodically. If P_{pool} is less than 85% of $P_{\text{individual}}$ ($P_{\text{pool}} < 0.85 \times P_{\text{individual}}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in “Laboratory Monitoring Part C” below.

- Alternatively, if the EUA test is a high sensitivity RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction), and has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel, size of pools may be increased taking into consideration Table 1, and the new n should not be more than the test developer validated maximum n in the EUA.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

B.2 If Historical Data for Individual Specimens is Unavailable

- After implementing a n-sample pooling strategy, first calculate the positivity rate ($P_{\text{pool-initial}}$) based on n-sample pool size using the data from testing pooled samples from the first 7-10 days*.
 - If $P_{\text{pool-initial}}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
 - If $P_{\text{pool-initial}}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{\text{pools-x}}$) for subsequent 7-10* day period based on n-sample pool testing. ($P_{\text{pool-x}}$) should be updated daily using a moving average.

Compare $P_{\text{pool-initial}}$ to $P_{\text{pool-x}}$ periodically. If $P_{\text{pool-x}}$ is less than 90% of $P_{\text{pool-initial}}$ ($P_{\text{pool-x}} < 0.90 \times P_{\text{pool-initial}}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in “Laboratory Monitoring Part C” below.
- Alternatively, if the EUA test is a high sensitivity RT-PCR assay which uses a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., silica bead extraction), and has established high sensitivity with an internationally recognized standard or FDA SARS-CoV-2 Reference Panel, size of pools may be increased taking into consideration Table 1, and the new n should not be more than the test developer validated maximum n in the EUA.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

* It is recommended that $P_{\text{individual}}$ be calculated from the previous 7-10 days, while P_{pool} and $P_{\text{pool-x}}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{\text{individual}}$, P_{pools} , and $P_{\text{pool-x}}$ may not be representative of the percent positivity in the testing

population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Sample Pooling Re-assessment (Laboratory Monitoring Part C)

Option 1: Stop n-sample pooling and return to individual testing

- Patient samples should be tested individually until 10 consecutive positive samples have been collected. The total number of samples, tested individually, depends on the positivity rate.
- Using these samples, 10 pools should be created and tested with 1 positive and (n-1) negative samples and the PPA between testing sample pools and individual samples should be calculated.

Individual testing	Study
Sample #1, Individual, negative	
Sample #2, Individual, negative	
Sample #3, Individual, positive	Convolute a pool with Sample#3 and (n-1) negative samples
Sample #4, Individual, negative	
.....	

- Alternatively, if the laboratory is using an EUA RT-PCR test that can generate Ct values, the laboratory may be able to assess PPA (EUA Test_{pool} vs. EUA Test_{individual}) in silico based on the individually tested sample results without performing any testing of pooled samples. In order to perform this in silico PPA assessment, the Instructions for Use (IFU) of this EUA test must include a reference table containing information regarding the in silico PPA (EUA Test_{pool} vs. EUA Test_{individual}) analysis rules established by the test developer for each test target for all validated pool sizes. Refer to the “Clinical Sample Pooling Validation Study at Three Geographically Diverse US Sites” section of the template for more information regarding this reference table.

Option 2: Continue n-sample pooling

- Re-assessment study should start from time T0 and should consist of simultaneous testing individual sample testing in parallel with the normal workflow of pooled testing. However, since all non-negative sample pools require individual testing of all individual samples included in the pool as a part of the n-sample pooling and deconvoluting workflow, the re-assessment study essentially consists of testing individual samples from the negative n-sample pools.
- Re-assessment study may pause at time T1 when a minimum of 10 consecutive positive individual results are obtained, including both positive individual results generated from individual testing of samples from the non-negative sample pools following the n-sample pooling and deconvoluting

workflow, and positive individual results obtained from individual testing of samples from the negative sample pools for the time period from T0 to T1 [T0, T1].

n-sample pools workflow	Study
Pool#1, negative	Deconvolute n individual samples
Pool#2, negative	Deconvolute n individual samples
Pool#3, negative	Deconvolute n individual samples
Pool#4, positive => n individual results	
.....	

- Considering that number of positive individual sample results among negative pools is K, PPA between testing n-sample pools and assaying single specimens using the candidate test should be calculated as PPA (EUA Test_{pool} vs. EUA Test_{individual}) = 100% x (10-K)/10. It is critical that all consecutive positive samples from time period [T0, T1] are included in the PPA calculations. With regard to calculating the PPA, all non-negative results testing pooled samples should be counted as in agreement with positive individually tested results.

Re-assessment Acceptance Criteria for Option 1 and Option 2

- If the PPA (EUA Test_{pool} vs. EUA Test_{individual}) is ≥ 90% (9 out of 10 or 10 out of 10), then implementation of testing using n-sample pooling is acceptable.
- If the PPA between pooled-testing results and individual-testing results is less than 90%:
 - If PPA ≤ 70% (7 out of 10), reduce the pool size (consider a new n as n-1)
 - If PPA is 80% (8 out of 10), collect an additional 10 consecutive individually positive samples. Then, calculate the PPA from the combined data of 20 samples, between pooled testing results and individual testing results. If the PPA is ≥ 85%, then implementation of testing using n-sample pooling is acceptable. Or, to compensate for lost sensitivity, reduce the pool size (consider a new n as n-1) and continue with the re-assessment testing until PPA of pooled compared to individual testing is ≥ 90%.
- If PPA of at least 85% cannot be reached for any pool size evaluated in the re-assessment, cease pooling patient specimens.

If n-sample pooling is acceptable based on re-assessment, re-establish P_{individual} in your laboratory by estimating the positivity rate from individual testing in the population from which the 10 (or 20) consecutive individual positive samples were collected. If the total number of samples (N*) that needed to be tested to obtain the 10 (or 20) consecutive positive samples is stopped at the 10th (or 20th) positive sample, then the positivity rate of 10/N* (or 20/N*) is overestimated. The positivity rate should be corrected by the following corresponding multiplier:

- Positivity rate for 10 samples is $(10/N^*) \times (10/11)$
- Positivity rate for 20 samples is $(20/N^*) \times (20/21)$.

This updated new positivity rate should be used as $P_{\text{individual}}$ in the future laboratory monitoring (return to section B.1 of the “Laboratory Monitoring Part B”).

Attachment 2 – Additional Label

Please print and place this label on the front panel of the QuantStudio 5 Real-Time PCR Instrument.

If the QuantStudio 5 Real-Time PCR Instrument includes labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The QuantStudio 5 Real-Time PCR Instrument should retain this labeling throughout the EUA use of the AMPIPROBE® SARS-CoV-2 Test System.

Emergency Use Only

**This instrument is authorized for use with
AMPIPROBE® SARS-CoV-2 Test System**

TROUBLESHOOTING

Category	Comments and Suggestions
General handling of GENFLEX®	During the run, if an error message appears, click “Repeat” and then click “Execute”. If error persists, click “Abort”.
Precipitation in reagents	Allow any precipitation to dissolve completely before loading the reagents into the deck of the GENFLEX® platform.
Magnetic particles are not completely resuspended.	Mix the AMPIXTRACT™ Magnetic Beads by vortexing before transferring the beads to the troughs. This ensures complete transfer of the beads to the troughs and better resuspension of the beads on the instrument.
Frozen samples are not mixed properly after thawing.	Mix the contents of the vial of the AMPIXTRACT™ Stabilizer with a manual pipette after thawing the product.
Degraded nucleic acids	All working surfaces should be cleaned with 10% bleach, followed by 70% ethanol solution before starting the extraction and PCR. Use RNase-free filtered pipette tips to prevent any introduction of RNases during the preparation of the Master Mix.
Incomplete sample lysis	Ensure that correct volume of ethanol is added to the AMPIXTRACT™ Lysis Reagent before loading onto the instrument.

SYMBOL DESCRIPTIONS



In vitro diagnostic medical device



Catalogue number



Batch code



Global Trade Item Number

Rx only

US Only: Federal law restricts this device to sale by or on the order of a physician.



Manufacturer



Use by date



Temperature limit



Consult instructions for use

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