Linea™ COVID-19

Linea™ COVID-19 Assay Kit

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For Emergency Use Authorization Only

Instructions for Use (IFU) v. 1.8

For *In-Vitro* Diagnostic (IVD) Use

For Prescription Use Only

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1. Intended Use

The Linea™ COVID-19 Assay Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens including anterior nasal swabs, self-collected at a healthcare location or collected by a healthcare worker, and nasopharyngeal and oropharyngeal swabs, mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens collected by a healthcare worker from individuals who are suspected of COVID-19 by their healthcare provider (HCP). The test is also intended for use with anterior nasal swab specimens that are self-collected in the presence of an HCP from individuals without symptoms or other reasons to suspect COVID-19 when tested at least weekly and with no more than 168 hours between serially collected specimens.

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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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 Linea™ COVID-19 Assay Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Linea™ COVID-19 Assay Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

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2. Summary and Explanation

The recent COVID-19 outbreak has a significant impact to healthcare and the economy in the U.S. and throughout the world. The secretary of U.S. Health and Human Services announced on January 31, 2020 that a Public Health Emergency Exists for SARS-CoV-2. The Linea™ COVID-19 Assay Kit uses the real-time reverse transcription polymerase chain reaction (rRT-PCR) to detect SARS-CoV-2 viral RNA. The product includes optimized oligonucleotide primers and probes (TaqMan®) specifically targeting target sequences within the Spike (S) gene of SARS-CoV-2, and control material used in rRT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens (such as nasopharyngeal swabs and oropharyngeal swabs, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and BALs. In this document, we have provided pertinent and timely information about the Linea™ COVID-19 Assay Kit.

3. Principles of the Assay

The Linea™ COVID-19 primer and probe sets are designed to detect RNA from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal swabs and oropharyngeal swabs, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and BALs from patients as recommended for testing by public health authority guidelines. The Linea™ COVID-19 assay uses primer and probe sets to detect two highly conserved sequences (S1 and S2) contained in SARS-CoV-2 Spike (S) Gene.

RNA from upper respiratory specimens is extracted using a validated RNA extraction system (QIAamp Viral RNA Mini Kit (Qiagen Cat. No.52906), Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Omega Cat. No. M6219-2304), Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the Hamilton STARlet system, Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the KingFisher Flex System, or Applied Biosystems MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit automated on the KingFisher Flex System) and if necessary transcribed to cDNA and subsequently amplified via rRT-PCR using a validated real-time PCR instrument. During the PCR amplification process, the probes anneal to specific target sequences 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 to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity signal is monitored at each PCR cycle by the real-time PCR instrument.

A sample is considered positive when the fluorescence intensity signal exceeds a predetermined baseline threshold value. The cycle number at which this occurs is referred to as the cycle threshold Ct. Detection of SARS-CoV-2 RNA in a sample is determined by the Ct value.

The Linea™ COVID-19 Assay Kit is designed to operate as a high-throughput assay run on 96-well plates, with 94-wells being available for patient specimen testing per 96-well plate. The Linea™ COVID-19 Assay Kit consists of reagent vials containing the primers and probes for the S1 and S2 sequence targets, positive controls and dilution solution. The contents of the reagent vials are transferred by the user to 96-well plates for patient specimen analysis pursuant to these Instructions for Use.

The Linea™ COVID-19 Assay Kit is designed to be used only with the Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ Dx Real-Time PCR system equipped with software v1.0.3 or the Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ 5 Real-Time PCR system equipped with QuantStudio Design and Analysis software v1.4, or the Applied Biosystems™ 7500 Fast Dx Real-Time PCR system.

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4. Material Provided

The Linea™ COVID-19 Assay Kit comes in the following kit sizes: 100 reactions, 500 reactions and 1000 reactions. For each kit size, the components included with the Linea™ COVID-19 Assay Kit are packaged in freestanding, sterile screw-top microcentrifuge vials as follows:

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- **Green Label**: S1 and S2 Primers and Probes
- **Red Label**: Positive Control (concentrated)
- **Orange Label**: Positive Control Dilution Buffer
- **Purple Label**: Master Mix
- **White Label**: Nuclease Free Water
- **Yellow Label**: RNase P Control

For each kit size, the kit vials contain the proper amounts of reagents for the designated kit reaction number in 0.1 mL 96-well plates. For each kit size, the vials contain the following volumes:

	100 Reactions (DX-1001-001-000)	500 Reactions (DX-1001-002-000)	1000 Reactions (DX-1001-003-000)
S1 and S2 primers and probes (green label)	0.1 mL	0.5 mL	1 mL
Positive control (concentrated) (red label)	0.05 mL	0.25 mL	0.5 mL
Positive control dilution buffer (orange label)	0.5 mL	2.5 mL	5 mL
Master Mix (purple label)	0.5 mL	2.5 mL	5 mL
Nuclease Free Water (white label)	1.5 mL	4.85 mL	9.7 mL
RNase P Control (yellow label)	0.03 mL	0.15 mL	0.3 mL

Prior to kit use, the concentrated positive control (red label) must be diluted with the positive control dilution buffer (orange label) according to Section 12.1.

5. Required Equipment and Consumables Not Provided

The Linea™ COVID-19 Assay Kit does NOT include the following materials:

Component Manufacture and Description	Catalog #	Manufacture
QIAamp® Viral RNA Mini Kit	52906	Qiagen GmbH
Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ Dx Real-Time PCR system	4480299	Thermo Fisher
Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ 5 Real-Time PCR system	A28138	Thermo Fisher
Applied Biosystems™ 7500 Fast Dx Real-Time PCR system	4406984	Applied Biosystems
MicroAmp™ Optical Adhesive Film	4311971	Thermo Fisher
MicroAmp™ Fast Optical 96-well Reaction Plate, 0.1 mL	4346907	Applied Biosystems
Omega Bio-Tek Mag-Bind® Viral RNA Xpress Kit	M6219-2304	Omega Bio-Tek

Hamilton Microlab STARlet Liquid Handling System	173000-034	Hamilton Company
Applied Biosystems MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383	Applied Biosystems
Thermo Fisher Scientific KingFisher™ Flex Purification System (software Bindit 4.0)	5400630	Thermo Fisher

The following RNA extraction systems have been validated for use with the Linea™ COVID-19 Assay Kit. Only one RNA extraction kit is necessary for kit use.

- QIAamp RNA extraction kit
- Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit
- Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the Hamilton STARlet System
- Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated on the Thermo Fisher KingFisher™ Flex Purification System
- Applied Biosystems MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit automated on the Thermo Fisher KingFisher™ Flex Purification System

6. Real-Time PCR Instrument

The following real-time PCR instruments have been validated for use with the Linea™ COVID-19 Assay Kit:

- Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ Dx Real-Time PCR system equipped with software v1.0.1 (Thermo Fisher Catalog No. 4480299)

- Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ 5 Real-Time PCR system equipped with QuantStudio Design and Analysis software v1.4 (Thermo Fisher Catalog No. A28138)
  - *See Appendix A for Emergency Use Only label that should be affixed to the exterior of the RUO labeled QuantStudio™ 5 for customer using this instrument

- Applied Biosystems 7500 Fast Dx Real-Time PCR system equipped with Applied Biosystems software v2.3 (Thermo Fisher Catalog No. 4406984)

Please ensure that all instruments used have been installed, calibrated and maintained according to the manufacturer's instruction and recommendations. Each real-time PCR system must be calibrated with ABY™ dye as per the manufacturer supplied Instructions for Use.

7. Facilities/Training Requirements

Clinical use of the Linea™ COVID-19 Assay Kit 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.

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Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures:

Refer to the Centers for Disease Control and Prevention (CDC) guidelines: Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2:

https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

In addition, refer to the World Health Organization Interim guidance on laboratory biosafety: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: interim guidance, March 2, 2020

https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117

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8. Warnings and Precautions

8.1 General

- For *in vitro* diagnostic use (IVD) only.
- For Emergency Use Authorization only.
- For prescription use only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) biological safety cabinet.

- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited) gloves, eye protection and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes and other equipment and reagents.
- Please consult the material safety data sheet (SDS) before using this kit, which is available on request.

8.2 Contamination

Amplification technologies such as RT-PCR are sensitive to accidental introduction of RT-PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicons).

The Linea™ COVID-19 Assay Kit positive control contains a high copy number of templates. It should be opened and processed away from clinical specimens and other kit components to avoid cross-contamination. In addition, you should:

- Maintain separate areas for handling of specimen preparation, pre-RT-PCR assay setup, and post-RT-PCR amplified nucleic acids.
- Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. microcentrifuge tubes, pipette tips) for handling of specimen preparation, pre-RT-PCR assay setup, and post-RT-PCR amplified nucleic acids.
- Wear a clean lab coat and disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Good aseptic technique should always be used when working with nucleic acids.
- When mixing reagents by pipetting up and down, this should be done with a volume roughly equal to 50% of the total component volume.

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- Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g. DNA/RNA remover, ethanol, 10% bleach) to minimize risk of nucleic acid contamination.
- Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
- Use DNase/RNase free disposable plastic ware and pipettes reserved for DNA/RNA work to prevent cross-contamination with DNases/RNases from shared equipment.
- Use DNase/RNase free filter tips throughout procedure to prevent aerosol and liquid contamination.

8.3 Nucleic Acid Extraction Systems

Please consult the relevant Instruction For Use (IFU) and Safety Data Sheets (SDS) available from the manufacturer before using one of the authorized RNA extraction systems, which include:

(i) Qiagen QIAamp;
(ii) Omega Bio-Tek Mag-Bind;
(iii) Omega Bio-Tek Mag-Bind automated on the Hamilton STARlet;
(iv) Omega Bio-Tek Mag-Bind automated on the ThermoFisher KingFisher Flex; or
(v) Applied Biosystems MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit automated on the Thermo Fisher KingFisher Flex.

Please note that only one RNA extraction system is necessary.

9. Reagent Storage, Handling and Stability Conditions

9.1 Storage Conditions

The Linea™ COVID-19 Assay Kit is shipped on dry ice and must be stored at -20 °C upon arrival. If the kit's protective packaging is damaged upon receipt, please contact Applied DNA Sciences for instructions. Attention should be paid to the expiration date specified on the pack label and individual tube labels. Once this date, the kit should be discarded following the disposal instructions in Section 13.

Always check the expiration date prior to use. Do not use expired reagents. Please protect fluorogenic primer/probe mix from light.

9.2 Stability

The Linea™ COVID-19 Assay Kit should be stored in the original packaging and is stable for up to 6 months stored at -20°C and should not be used past any expiration date indicated on the kit packaging.

When in use, the kit components should be returned to the freezer promptly after use to minimize the time at room temperature. Do not exceed 3 freeze/thaw cycles.

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10. Specimen Collection, Handling, Transport and Storage

Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of the Linea™ COVID-19 Assay Kit. Improper collection, storage, or transport of specimens may lead to false negative results. For details, refer to the CDC guideline "Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)" https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

11. RNA Extraction

The results of the Linea™ COVID-19 Assay Kit are dependent upon the amount and quality of template RNA purified from human specimens. No RNA extraction kit or system is supplied with the Linea™ COVID-19 Assay Kit.

The Linea™ COVID-19 assay is validated for use with the below RNA extraction kits or systems. Follow the manufacturer supplied Instructions for Use for the chosen RNA extraction kit or system.:

- Qiagen GmbH QIAamp Viral RNA Mini Kit (Cat. No. 52906)
- Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Cat. No. M6129-2304)
- Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Cat. No. M6129-2304) automated on the Hamilton STARlet System (Cat. No. 173000-034).
- Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Cat. No. M6129-2304) automated on the Thermo Fisher KingFisher Flex System (Cat. No. 5400630).
- Applied Biosystems MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383) automated on the Thermo Fisher KingFisher Flex System (Cat. No. 5400630).

For the chosen RNA extraction kit, final elution volume should be 50 µL.

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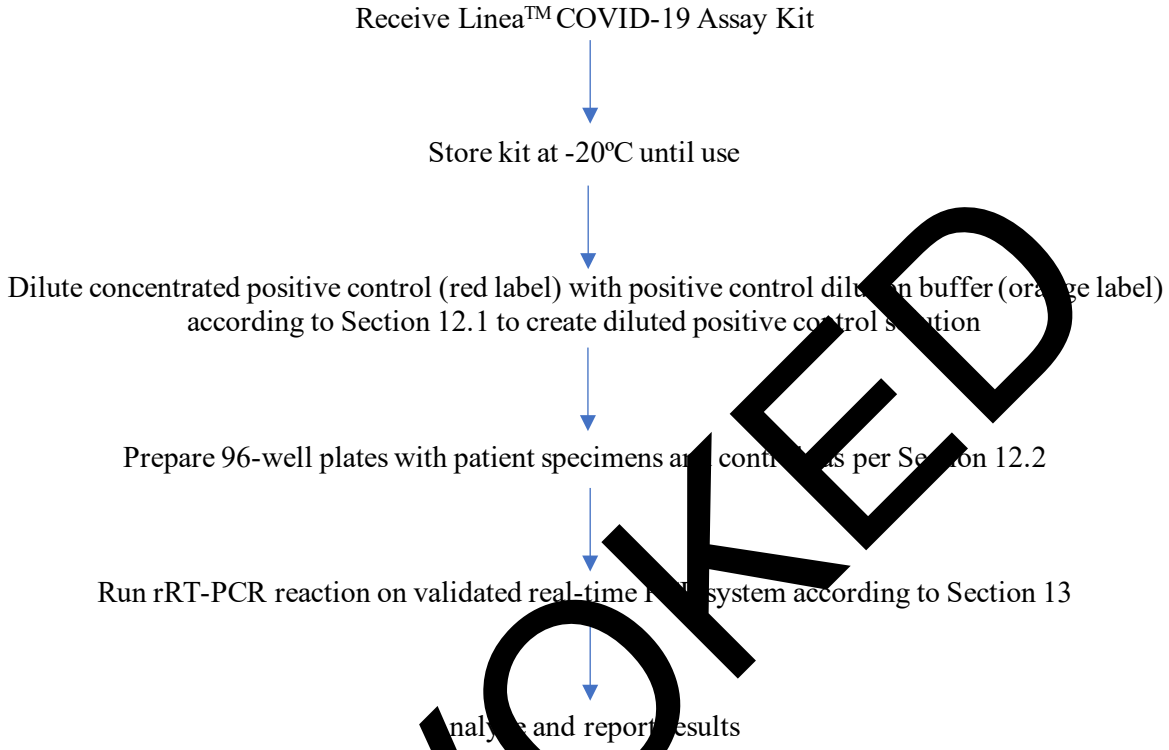
12. rRT-PCR Reaction Setup

After RNA extraction, the purified nucleic acid is reverse transcribed and amplified using the Linea™ COVID-19 Assay Kit. An overview workflow is provided below:



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12.1 Preparation of Positive Control

The Linea™ COVID-19 Assay Kit concentrated positive control (**red label vial**) must be diluted with the positive control dilution buffer (**orange label vial**) prior to the rRT-PCR reaction as follows:

- Pipet 98 µL of positive control dilution buffer (orange label) into a microcentrifuge tube, then add 2 µL of concentrated positive control (red label). Mix well, then centrifuge briefly.
- Pipet another 187.5 µL of positive control dilution buffer (orange label) into a second microcentrifuge tube, then add 12.5 µL of the dilution created in the step above. Mix well, then centrifuge briefly. Final volume is 100 µL.

12.2 Preparation of 96-well plates

Well Contents

The assay is configured for use with a MicroAmp™ Fast Optical 96-well Reaction Plate, 0.1mL (Applied Biosystems Cat. No. 4346907) or similar 96-well plate.

On each plate, up to 94 wells are available for patient specimens, while 2 wells are used for controls (1 positive control and 1 no template control (NTC)). Each of the up to 94 wells used for patient

specimen testing contain the reaction mix listed in Table 1. The positive and NTC well reagent compositions are listed in Table 2 and Table 3, respectively.

Table 1: Volume of Linea™ COVID-19 Assay Kit Reagents per Specimen Well for 96-well plate

Reagent	Volume per Sample (µL)
Master Mix - **Purple Label Vial**	5.0
S1 and S2 Primers and Probes - **Green Label Vial**	1.0
RNase P Control – **Yellow Label Vial**	0.3
Nuclease-free Water – **White Label Vial**	9.7
Purified Nucleic Acid Specimen	4.0
Total Reaction Mix Volume Per Well	**20.0**

Table 2: Volume of Linea™ COVID-19 Assay Kit Reagents for Positive Control Well

Reagent	Volume per Sample (µL)
Master Mix - **Purple Label Vial**	5.0
S1 and S2 Primers and Probes- **Green Label Vial**	1.0
RNase P Control – **Yellow Label Vial**	0.3
Nuclease-free Water – **White Label Vial**	9.7
Positive Control - **From Step 12.1**	4.0
Total Reaction Mix Volume Per Well	**20.0**

Table 3: Volume of Linea™ COVID-19 Assay Kit Reagents for NTC (No Template Control)

Reagent	Volume per Sample (µL)
Master Mix - **Purple Label Vial**	5.0
S1 and S2 Primers and Probes- **Green Label Vial**	1.0
RNase P Control – **Yellow Label Vial**	0.3
Nuclease-free Water – **White Label Vial**	13.7
Total Reaction Mix Volume Per Well	**20.0**

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Create a 96-well Plate for Linea™ COVID-19 Assay

To create a 0.1mL 96-well plate with the volumes listed in Tables 1-3 perform the following two-step preparation:

Step 1: Prepare Reaction Mix according to the below table:

Component	Total volume for 94 sample wells and 2 control wells (µL)
Master Mix - **Purple Label Vial**	528
S1 and S2 Primers and Probes- **Green Label Vial**	105
RNase P Control – **Yellow Label Vial**	[illegible]7
Nuclease-free Water – **White Label Vial**	1024.3
Total Reaction Mix volume	1689.6

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Step 2: Set up the 96-well plate:

Pipette 16.0 µL of the Reaction Mix prepared in Step 1 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL. Then combine with the purified nucleic acid specimen or control according to the following table.

Component	Volume per well (µL)		
	Specimen Wells (94)	Positive Control Well (1)	NTC Well (1)
Reaction Mix prepared in **Step 1**	16.0	16.0	16.0
Purified Nucleic Acid Specimen	4.0	—	—
Diluted positive control solution - **From Step 12.1**	—	4.0	—
Nuclease-free Water – **White Label Vial**	—	—	4.0
Total Volume Per Well	20.0	20.0	20.0

13. Programing the Real-Time PCR Instrument

Please refer to manufacturer supplied user manual for the Thermo Fisher Scientific QuantStudio™ Dx, Thermo Fisher QuantStudio™ 5 or the Applied Biosystems™ 7500 Fast Dx Real-Time PCR systems for how to set an amplification program. A report must issue with Ct values for all wells.

Set the following detection targets for each well in 96-well plate:

Reporter dye/Quencher	Detector
VIC / NFQMGB	S1 Target
FAM / NFQMGB	S2 Target
ABY/ QSY (none)	RNase P

Important – The passive reference must be set to ROX.

Enter into the instrument the following amplification program:

Table 4: PCR Thermal Cycling Conditions on Validated Real-Time PCR Instruments

Temperature	Time	Cycle	Reaction
50°C	5 min	1	Reverse Transcription
95°C	20 sec		Holding
95°C	3 sec	45	Amplification
60°C	30 sec		

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14. Interpretation of Results

14.1 Controls – Positive and NTC

Table 5 contains the expected performance of the controls included in the Linea™ COVID-19 Assay Kit.

Table 5: Interpretation of Results For No Template and Positive Control Reactions

Control	S1 (VIC)	S2 (FAM)	RNase P (ABY)	Expected Ct Value	Used to Monitor
No Template Control		-	-	Not detected	Reagent and/or environmental contamination
Positive Control	+	+	-	<40	Reagent failure including primer/probe integrity

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test from the extracted samples.

14.2 Interpretation of Patient Specimens

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Table 6 below shows the interpretation of patient specimens. If results are obtained that do not adhere to these guidelines, re-extract and retest the existing specimen. If one or more controls are not valid, the patient results cannot be interpreted.

Table 6: Interpretation of Patient Specimen Results Using Linea™ COVID-19 Assay Kit

S1 Ct <40 (VIC)	S2 Ct <40 (FAM)	RNase P Ct <40 (ABY)	Interpretation	Report Result	Actio
+	+	+	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities
-	+	+	SARS-CoV-2 is **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities
+	-	+	SARS-CoV-2 is **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities
-	-	+	SARS-CoV-2 **Detected**	**NEGATIVE**	Reported to sender and appropriate public health authorities
+/-		-	**Invalid Result**	**INVALID**	Repeat extraction and RT-PCR. If the repeated result remains INVALID, consider collecting a new specimen from the patient, if clinically indicated.

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15. Limitations

- Negative results do not preclude infection with SARS-CoV-2 and should not be used as the sole basis of a patient treatment/management decision. All results should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms.

- Interpretation of rRT-PCR test results must account for the possibility of false negative and false positive results. False negative results can arise from:
    - poor sample collection or
    - degradation of the viral RNA during shipping or storage or
    - specimen collection conducted prior to symptom onset
    - failure to follow the authorized assay procedures
    - failure to use authorized extraction kit and instrument
- False positive results can arise from:
    - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
    - Unsuitable handling of amplified product.
- The performance of the Linea™ COVID-19 Assay Kit was established using RNA extracted from previously collected clinical nasopharyngeal and oropharyngeal swabs only.
- Healthcare worker collected nasopharyngeal washes/aspirates or nasal aspirates, mid-turbinate nasal swabs, and bronchoalveolar lavage (BAL) samples, and self-collected or healthcare worker collected anterior nasal swabs are additional acceptable respiratory specimens that can be tested with the Linea™ COVID-19 Assay Kit; however, performance with these specimen types has not been determined.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. Improper collection, storage, or transport of specimens may lead to false negative results.
- The impact of the administration of SARS-CoV-2 vaccines and/or therapeutics on the ability to detect SARS-CoV-2 RNA in patient specimens has not been evaluated.
- The presence of RT-PCR inhibitors may cause false negative or invalid results
- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the S1 or S2 primer sets of the Linea™ COVID-19 Assay Kit. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- This test cannot rule out disease by other pathogens.
- Based on *in silico* analysis and clinical observation, the Linea™ COVID-19 Assay Kit experiences reduced sensitivity of the S1 target in the presence of the 69-70del SARS-CoV-2 S-gene mutation. The 69-70del mutation has been observed in SARS-CoV-2 variants within the United States and other countries. The 69-70del mutation is a 6-nucleotide deletion (21765-21770) within the S-gene that results in the deletion of two amino acids at sites 69 and 70 in the spike protein. The 69-70del mutation has been

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observed in multiple variant lineages of SARS-CoV-2, including the B.1.1.7 variant (UK VOC-202012/01/Alpha). Detection of the Linea™ COVID-19 Assay Kit's S2 target is not impacted by the 69-70del mutation. Therefore, the reduction in S1 target sensitivity in the presence of the 69-70del mutation is unlikely to result in a decrease in the overall sensitivity of the Linea™ COVID-19 Assay Kit. The Interpretation of Patient Specimens as provided in Section 14.2 of this IFU provides for a positive result to be called if at least one of the two assay targets is detected. Since there is no 69-70del mutation related impact on the Linea™ COVID-19 Assay Kit's S2 target, a sample containing the 69-70del mutation can be called positive based on detection of the S2 target.

- Based on *in silico* analysis, it was determined that the P681H mutation, a single-nucleotide substitution from "C" to "A" at nucleotide 23604 of the SARS-CoV-2 genome, falls within the region of the SARS-CoV-2 genome targeted by the Linea™ COVID-19 Assay Kit's S2 target probe sequence. Based on both clinical observations and wet testing results with reference material containing the P681H mutation, the analytical sensitivity of the S2 assay target is not impacted by the P681H single-nucleotide substitution.

- The clinical performance has not been established for 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.

- The performance of the Linea™ COVID-19 Assay Kit has not yet been clinically validated for use in patients without signs and symptoms of respiratory infection, or for serial screening applications and performance may differ in these populations.

- Asymptomatic individuals infected with COVID-19 may not shed enough virus to reach the limit of detection of the test, giving a false negative result.

- In the absence of symptoms, it is difficult to determine if asymptomatic individuals have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.

- The clinical performance has been established with specimens collected from subjects suspected of COVID-19 or subjects suspected of COVID-19 presenting with one or more symptoms of COVID-19. Performance with specimens collected from individuals without symptoms or other epidemiological reasons to suspect COVID-19 has not been established; a study to determine the performance in individuals without symptoms or other epidemiological reasons to suspect COVID-19 will be completed.

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16. Conditions of Authorization for the Laboratory

The Linea™ COVID-19 Assay Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the Linea™ COVID-19 Assay Kit, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the Linea™ COVID-19 Assay Kit 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.

B. Authorized laboratories using the Linea™ COVID-19 Assay Kit must use the product as outlined in the "Linea™ COVID-19 Assay Kit Instructions for Use". Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive the Linea™ COVID-19 Assay Kit must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using the Linea™ COVID-19 Assay Kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of the Linea™ COVID-19 Assay Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Applied DNA Sciences (via email: dxcovid@adnas.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the product of which they become aware.

F. All laboratory personnel using the Linea™ COVID-19 Assay Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

G. Applied DNA Sciences, authorized distributors, and authorized laboratories using the Linea™ COVID-19 Assay Kit must ensure that any records associated with the EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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[1] 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."

17. Performance Evaluation

The Linea™ COVID-19 Assay Kit performance evaluation has been generated only on the following real-time PCR instruments:

- Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ Dx Real-Time PCR System equipped with software v1.0.3 (Thermo Fisher Catalog No. 4480299)
- Thermo Fisher Scientific (Applied Biosystems) QuantStudio™ 5 Real-Time PCR System equipped with QuantStudio Design and Analysis software v1.4 (Thermo Fisher Catalog No. A28138)
- Applied Biosystems™ 7500 Fast Dx Real-Time PCR system equipped with Applied Biosystems Software v 2.3 (Thermo Fisher Catalog No. 4406984)

17.1 Analytical Sensitivity (LoD)

Limit of detection (LoD) studies determine the lowest detectable concentration of the SARS-CoV-2 virus at which approximately 95% of all true positive replicates test positive for both the S1 and S2 targets. The LoD was determined using quantified viral inactivated SARS-CoV-2 RNA obtained from ATCC, Manassas, VA (ATCC VR-1986D). Spiked samples were created by serial dilutions of whole viral RNA spiked into pooled clinical nasopharyngeal swab matrix and extracted with the QIAamp method. The spiked samples were tested using either 21 or 24 individual extraction replicates per concentration on a QuantStudio™ Dx with the Linea™ COVID-19 Assay Kit.

As shown in Table 7, the LoD confirmatory study results show that the LoD of the Linea™ COVID-19 Assay Kit is 5 copies per reaction (1.25 copies/µL) for nasopharyngeal specimens using the QIAamp extraction kit.

Table 7: LoD Results of Linea™ COVID-19 Assay Kit Using ATCC RNA Spiked in QIAamp RNA Extracted Matrix

Copies per Reaction	Copies per µL	S1 Target			S2 Target		
		Positive Replicates	Average Ct	Standard Deviation	Positive Replicates	Average Ct	Standard Deviation
40	10	21/21	33.1	1.7	21/21	33.9	0.31
30	7.5	24/24	34.5	0.53	24/24	34.6	0.24
20	5	24/24	34.1	1.9	24/24	35.0	0.36
10	2.5	24/24	36.3	0.84	24/24	36.4	0.61
5	**1.25**	**21/21**	**35.6**	**1.3**	**21/21**	**35.9**	**0.96**
2.5	0.625	21/21	36.9	0.7	17/21	36.9	0.66

An additional LoD study for the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit was performed using quantified heat inactivated SARS-CoV-2 virus obtained from ATCC, Manassas, VA (VR-1986HK) spiked into pooled negative clinical nasopharyngeal matrix. 10-fold serial dilutions of the inactivated SARS-CoV-2 virus were spiked into pooled negative clinical nasopharyngeal matrix and extracted with the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit to obtain the LoD range.

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Once the initial LoD range was established, confirmation of the final LoD was determined using 2-fold serial dilutions of inactivated whole SARS-CoV-2 virus spiked in to pooled negative clinical nasopharyngeal matrix. 20 extraction replicates were tested at each dilution level on the QuantStudio™ Dx Real-Time PCR system. As shown below, the final LoD of the Linea™ COVID-19 Assay Kit utilizing the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit was determined to be 2.5 copies per reaction (0.625 copies/µL) for nasopharyngeal specimens.

Table 8: LoD Results of Linea™ COVID-19 Assay Kit Using Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Manual Extraction) on the QuantStudio™ Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
10	2.5	20/20	34.2	20/20	34.1
5	1.25	20/20	34.9	20/20	35.1
2.5	**0.625**	**20/20**	**35.3**	**20/20**	**35.7**
1.25	0.313	20/20	36.2	17/20	36.3
0.625	0.156	18/20	37.1	12/20	37.2

An abridged bridging study was also conducted for use of quantified heat inactivated SARS-CoV-2 virus obtained from ATCC, Manassas, VA (VR-1986/HK) as a reference material for LoD determinations for the Linea™ COVID-19 Assay Kit. The quantified heat inactivated SARS-CoV-2 virus was spiked into pooled negative clinical nasopharyngeal matrix at a concentration of 1.25 copies/µL. 20 replicates were extracted using the Qiagen kit and 20 replicates were extracted using the Omega Mag-Bind kit. The results of the study are summarized below in Table 9.

Table 9: Summary results of abridged bridging study for quantified heat inactivated SARS-CoV-2 virus reference material spiked at 1.25 copies/µL

Extraction Kit	S1 Target		S2 Target	
	Positive Replicates	Average Ct	Positive Replicates	Average Ct
Qiagen QIAamp	20/20	36.3	20/20	35.8
Omega Mag-Bind	20/20	34.9	20/20	35.6

Additional LoD studies of the Linea™ COVID-19 Assay Kit were conducted utilizing the QuantStudio™ 5 Real-Time PCR System. The studies established the LoD of the Linea™ COVID-19 Assay Kit utilizing the QuantStudio™ 5 Real-Time PCR System, which was confirmed by testing 21 replicates. As shown in Tables 10 and 11, the LoD confirmatory study results show that the LoD of the Linea™ COVID-19 Assay Kit utilizing the QuantStudio™ 5 Real-Time PCR System is 5 copies per reaction (1.25 copies/µL) for nasopharyngeal specimens using both the Omega Mag-Bind and QIAamp extraction kits.

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Table 10: LoD Results of Linea™ COVID-19 Assay Kit Using Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Manual Extraction) on the QuantStudio™ 5 Real-Time PCR System

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
10	2.5	21/21	34.7	21/21	34.1
5	**1.25**	**21/21**	**35.6**	**20/21**	**35.9**
2.5	0.625	21/21	36.2	17/21	36.9

Table 11: LoD Results of Linea™ COVID-19 Assay Kit Using Qiagen QIAamp Viral RNA Mini Kit (Manual Extraction) on the QuantStudio™ 5 Real-Time PCR System

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
10	2.5	21/21	36.0	21/21	36.6
5	**1.25**	**21/21**	**36.8**	**21/21**	**36.3**
2.5	0.625	21/21	37.5	18/21	37.7

LoD studies were also conducted to validate the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated by the Hamilton STARlet system for use with the Linea™ COVID-19 Assay Kit. The studies established the LoD of the Linea™ COVID-19 Assay Kit utilizing the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated by the Hamilton STARlet system to be 2.5 copies per reaction (0.625 copies/µL) for the QuantStudio™ Dx and 5 copies per reaction (1.25 copies/µL) for the QuantStudio™ 5 for nasopharyngeal specimens.

Table 12: LoD Results of Linea™ COVID-19 Assay Kit Using Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the Hamilton STARlet System on the QuantStudio Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	21/21	33.9	21/21	33.8
10	2.5	21/21	34.8	21/21	34.5
5	1.25	21/21	35.8	21/21	35.5
2.5	**0.625**	**20/21**	**36.8**	**21/21**	**36.5**
1.25	0.3125	21/21	37.4	18/21	36.9

Table 13: LoD Results of Linea™ COVID-19 Assay Kit Using Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the Hamilton STARlet System on the QuantStudio 5

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
20	5	21/21	33.9	21/21	33.2
10	2.5	21/21	34.7	21/21	33.7
5	**1.25**	**21/21**	**35.7**	**21/21**	**35.1**
2.5	0.625	21/21	36.8	19/21	35.4
1.25	0.3125	21/21	37.4	19/21	36.3

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Further LoD studies were conducted to validate the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit automated by the Thermo Fisher KingFisher Flex System and the Applied Biosystems MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit automated by the Thermo Fisher KingFisher Flex System for use with the Linea™ COVID-19 Assay Kit. The results of these LoD studies are shown in Tables 14 - 19.

Table 14: LoD Results of Linea COVID-19 Assay Kit Using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems QuantStudio 5 Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40	10	20/20	32.5	20/20	32.0
20	5	20/20	33.4	20/20	33.2
10	2.5	20/20	34.6	20/20	34.0
5	1.25	20/20	35.5	20/20	35.6
2.5	0.625	20/20	36.6	20/20	36.5
1.25	**0.3125**	**19/20**	**37.1**	**19/20**	**37.1**
0.625	0.15625	16/20	37.7	17/20	38.3

Table 15: LoD Results of Linea COVID-19 Assay Kit Using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems QuantStudio Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40	10	20/20	32.5	20/20	32.9
20	5	20/20	33.9	20/20	33.7
10	2.5	20/20	34.8	20/20	34.9
5	1.25	20/20	35.7	20/20	36.0
2.5	**0.625**	**20/20**	**36.6**	**20/20**	**37.3**
1.25	0.3125	20/20	37.9	15/20	38.1

Table 16: LoD Results of Linea COVID-19 Assay Kit Using the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems 7500 Fast Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40	10	20/20	32.0	20/20	32.6
20	5	20/20	32.9	20/20	33.4
10	2.5	20/20	34.2	20/20	34.8
5	1.25	20/20	35.2	20/20	35.6
2.5	0.625	20/20	36.0	20/20	36.5
1.25	**0.3125**	**20/20**	**36.7**	**20/20**	**37.9**
0.625	0.15625	16/20	38.1	16/20	38.7

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Table 17: LoD Results of Linea COVID-19 Assay Kit Using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems QuantStudio 5 Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40	10	20/20	32.3	20/20	32.1
20	5	20/20	33.3	20/20	33.0
10	2.5	20/20	34.2	20/20	33.8
5	1.25	20/20	35.3	20/20	34.6
2.5	0.625	20/20	36.4	20/20	35.9
1.25	0.3125	20/20	37.3	19/20	36.8
0.625	**0.15625**	**20/20**	**37.7**	**19/20**	**37.3**
.3125	0.007813	13/20	37.9	12/20	37.9

Table 18: LoD Results of Linea COVID-19 Assay Kit Using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems QuantStudio Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40	10	20/20	32.2	20/20	32.4
20	5	20/20	33.1	20/20	33.4
10	2.5	20/20	34.1	20/20	34.3
5	1.25	20/20	35.1	20/20	35.2
2.5	0.625	20/20	36.2	20/20	36.6
1.25	**0.3125**	**20/20**	**37.4**	**19/20**	**37.9**
0.625	0.15625	18/20	38.0	17/20	38.5

Table 19: LoD Results of Linea COVID-19 Assay Kit Using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit Automated on the KingFisher Flex Purification System on the Applied Biosystems 7500 Fast Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40		20/20	32.5	20/20	32.7
20		20/20	33.4	20/20	33.5
10	2.5	20/20	34.5	20/20	34.4
5	1.25	20/20	35.3	20/20	35.3
2.5	0.625	20/20	36.7	20/20	36.7
1.25	**0.3125**	**20/20**	**37.7**	**19/20**	**37.8**
0.625	0.15625	16/20	38.4	16/20	37.9

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A further LoD study was performed to validate the Applied Biosystems 7500 Fast Dx Real-Time PCR system for use with the Linea™ COVID-19 Assay Kit. The study utilized the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit and quantified heat inactivated SARS-CoV-2 virus spiked into pooled negative nasopharyngeal matrix. As shown in Table 20, the study established the LoD of the Linea™ COVID-19 Assay Kit utilizing the Applied Biosystems 7500 Fast Dx Real-Time PCR system to be 5 copies per reaction (1.25 copies/µL) for nasopharyngeal specimens using at least 20 individual extraction replicates.

Table 20: LoD Results of Linea™ COVID-19 Assay Kit Using Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit (Manual Extraction) on the Applied Biosystems 7500 Fast Dx Real-Time PCR system

Copies per Reaction	Copies per µL	S1 Target		S2 Target	
		Positive Replicates	Average Ct	Positive Replicates	Average Ct
40	10	21/21	33.6	21/21	32.[illegible]
20	5	22/22	34.8	[illegible]/22	[illegible].8
10	2.5	22/22	35.4	22/22	35.7
5	**1.25**	**22/22**	**36.1**	**22/22**	**35.9**
2.5	0.625	22/22	36.9	[illegible]/22	37.0

In addition, based on *in silico* analysis, it was determined that the P681H mutation, a single-nucleotide substitution from "C" to "A" at nucleotide [illegible] of the SARS-CoV-2 genome, falls within the region of the SARS-CoV-2 genome targeted by the Linea™ COVID-19 Assay Kit's S2 assay target probe sequence. To confirm that there is no impact on the S2 assay target's analytical sensitivity caused by the P681H mutation, a LoD study for the S2 assay target was conducted utilizing heat-inactivated quantified whole SARS-CoV-2 of the B.1.1.7 (Alpha) lineage (BEI Resources Cat No. NR-55245, isolate USA/CA_CDC_5574/202, GISAID Accession ID: EPI_ISL_751801) that contains the P681H single-nucleotide substitution. The LoD study was conducted using the combination of the Qiagen QIAamp RNA Kit for nucleic acid extraction and the QuantStudio 5 Real-Time PCR instrument according to the Linea™ COVID-19 Assay Kit's Instructions for Use. For the LoD study the heat-inactivated quantified B.1.1.7 SARS-CoV-2 virus was spiked into negative nasopharyngeal swab matrix and 20 extraction replicates were tested at each dilution level to determine the final LoD.

As shown in Table 21, the LoD study results showed that the analytical sensitivity of the S2 assay target is not impacted by the P681H single-nucleotide substitution.

Table 21. LoD results of Linea™ COVID-19 Assay Kit S2 Target in Samples with the P681H Mutation*

Copies per Reaction	Copies per µL	S2 Target		
		Positive Replicates	Average Ct	Standard Deviation
10	2.5	20/20	36.12	0.54
5	1.25	20/20	36.75	0.78
2.5	**0.625**	**19/20**	**38.26**	**0.83**
1.25	0.3125	17/20	38.85	0.75

* The LoD of the S1 target was not determined since the P681H mutation is specifically located in the S2 target.

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17.2 Analytical Specificity

Inclusivity

In silico evaluation of the S1 and S2 primers and probes was updated on January 25, 2021 using the 33,361 complete SARS-CoV-2 genomes in the NCBI/GenBank database. The *in silico* inclusivity analysis generated the following data:

Target	Primer/Probe	Percent Identities
S1	Forward Primer	99.4% (33,178/33,361)
	Reverse Primer	100%
	Probe	99.4% (33,171/33,361)
S2	Forward Primer	100%
	Reverse Primer	100%
	Probe	100%

According to NCBI data, the S2 forward and reverse primers, S2 probe and S1 reverse primer each showed 100% identity to the evaluated sequences. The S1 forward primer and S1 probe showed 100% identity to 99.4% of the evaluated sequences.

The main reason for the lower homology of the S1 forward primer and S1 probe is the recent emergence of circulating SARS-CoV-2 variants that contain the 69-70del SARS-CoV-2 S-gene mutation. Additional *in silico* analysis showed that variants containing the 69-70del mutation cause a misalignment of the Linea™ COVID-19 Assay Kit's S1 forward primer and S1 probe, resulting in reduced analytical sensitivity for the S1 assay target. This reduction in S1 target sensitivity has also been observed in testing of clinical samples with the Linea™ COVID-19 Assay Kit. The sensitivity of the Linea™ COVID-19 Assay Kit's S2 target is not impacted by the 69-70del mutation. The 69-70del mutation has been observed in SARS-CoV-2 variants within the United States and worldwide. The 69-70del mutation is a 6-nucleotide deletion (21765-21770) within the S-gene that results in the deletion of two amino acids at sites 69 and 70 in the spike protein. The 69-70del mutation has been observed in multiple variant lineages of SARS-CoV-2, including the B.1.1.7 variant (UK VOC-202012/01).

To ascertain the potential probability of 69-70del mutation related S1 target reduction in sensitivity for the Linea™ COVID-19 Assay Kit, the percentage of publicly available high coverage complete genome sequences on the Global Initiative on Sharing All Influenza Data (GISAID) containing the 69-70del mutation was calculated. As of January 21, 2021, GISAID contained 280,220 high coverage complete SARS-CoV-2 genome sequences (only entries with <1% Ns and <0.05% unique amino acid mutations and no insertions/deletions unless verified), of which, 5,239 (approximately 1.9%) contain the 69-70del mutation.

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The reduction in S1 target analytical sensitivity in the presence of the 69-70del mutation is unlikely to result in a decrease in the clinical sensitivity of the Linea™ COVID-19 Assay Kit. The Interpretation of Patient Specimens as provided in Section 14.2 of this IFU indicates that a positive result is reported if at least one of the two assay targets is detected. Since there is no 69-70del mutation related impact on the Linea™ COVID-19 Assay Kit's S2 target, the impact of the 69-70del mutation on the Linea COVID-19 Assay Kit's clinical performance is likely to be negligible.

Cross-Reactivity

In silico analysis was conducted for the Linea™ COVID-19 Assay Kit primers and probes utilizing the NIH NCBI BLAST sequence alignment system. The pairwise sequence alignment verifies the number of sequence matches between the primers and probes of the Linea™ COVID-19 Assay Kit and the target organism listed in Table 21. Anything less than an 80% match of the primers and probe is categorized as non-cross-reactive. The organism tested and results of the analysis are outlined in Table 22, below:

Table 22: Results of *in silico* Cross-Reactivity Study

Sample Name	S gene Target 1	S gene Target 2	Notes
Adenovirus 11	None	None	
Adenovirus 5	None	None	
Bordetella pertussis	None	None	
Chlamydophila pneumoniae	None	None	
Enterovirus 68	None	None	
Haemophilus influenzae	None	None	
Human coronavirus 229E	None	None	
Human coronavirus OC43	None	None	
Human coronavirus HKU1	None	None	
Human coronavirus NL63	None	None	
Human metapneumovirus	None	None	
Human parainfluenza virus 1	None	None	
Human parainfluenza virus 2	None	None	
Human parainfluenza virus 3	None	None	
Human parainfluenza virus 4b	None	None	
Human respiratory syncytial virus	None	None	
Human rhinovirus 61	None	None	
Influenza A	None	None	
Influenza B	None	None	
Legionella pneumophila	None	None	
Middle East Respiratory Syndrome coronavirus	None	None	
Mycobacterium tuberculosis	None	None	
Mycoplasma pneumoniae	None	None	
Severe Acute Respiratory Syndrome coronavirus (SARS-1)	**	None	S1 probe has >80% homology, but no amplification from Forward and Reverse primers.
Streptococcus pneumoniae	None	None	

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Sample Name	S gene Target 1	S gene Target 2	Notes
Streptococcus pyogenes	None	None	
Pneumocystis jirovecii	None	None	
Candida albicans	None	None	
Pseudomonas aeruginosa	None	None	
Staphylococcus epidermidis	None	None	
Streptococcus salivarius	None	None	

**Result returned >80 homology

Microbial Interference Studies: As noted in Table 21 above, the only sequence with substantial similarity (>80% homology) was SARS-1 with the S1 probe. The *in silico* analysis found no substantial similarity between the S1 forward and reverse primers and the SARS-1 genome and thus no amplification of the SARS-1 genome will occur. This fact eliminates any chance of detectable off target S1 probe binding. In addition, there are no known current infection of SARS-1 in the human population. Historical infections have been limited only to Asia. As such, it is not expected that the S1 probe's substantial similarity with the SARS-1 genome will impact the clinical utility of the Linea™ COVID-19 Assay Kit.

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17.3 Clinical Performance Evaluation

Performance of the Linea™ COVID-19 Assay Kit was evaluated using 60 deidentified individual clinical nasopharyngeal specimens that were previously tested using an EUA authorized molecular assay in a CLEP/CLIA-approved University Hospital Pathology Laboratory. Of these 60 clinical specimens 30 tested positive for SARS-CoV-2 RNA and 30 tested negative for SARS-CoV-2 RNA as per the authorized comparator assay. For clinical evaluation of the Linea™ COVID-19 Assay Kit, specimens were extracted using the QIAamp RNA extraction kit. All samples were blinded and randomized for testing on the Thermo Fisher Scientific QuantStudio™ Dx Real-Time PCR system. The results of the clinical evaluation are displayed below in Table 23.

Table 23: Results of Linea™ COVID-19 Assay Kit Against an Authorized Molecular Comparator Method with QIAamp Extraction

		EUA Authorized Molecular Comparator Assay		
		Positive	**Negative**	**Total**
Linea™ COVID-19 Assay Kit	**Positive**	29	1	**30**
	Negative	1	29	**30**
	Total	**1**	**29**	**60**
Positive Percent Agreement		**97%* (29/30)**		
Negative Percent Agreement		**97%** (29/30)**		

*95% Confidence Interval: 82.8% - 99.9%
**95% Confidence Interval: 82.8% - 99.9%

Positive percent agreement and negative percent agreement were both 97% (29/30 specimens) due to one false positive sample and one false negative sample. The Linea™ COVID-19 Assay Kit detected S1 and/or S2 target RNA in one clinical specimen in which the authorized molecular comparator assay failed to detect SARS-CoV-2 RNA. According to the Ct value for the one false positive, this discordant result showed a high Ct value (> 35) with the Linea™ COVID-19 Assay Kit, indicating that this sample was a weak positive. No additional discordant analyses were completed for the one false positive and one false negative result.

17.4 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. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the QIAamp Viral RNA Mini Kit and QuantStudio™ Dx Real-Time PCR system, respectively. The results are summarized in Table 24.

Table 24: 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	Nasopharyngeal	2.5x10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

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18. Disposal

Dispose of unused kit reagents, human specimens and sealed post-amplification plates according to local, state and federal regulations.

19. Quality Control

In accordance with Applied DNA's Standard Operating Procedure and Quality Control Program, each batch of the Linea™ COVID-19 Assay Kit is tested against predetermined specifications to ensure consistent product quality.

20. Technical Assistance

For customer support, please contact our dedicated technical support team:

Email: dxcovid@adnas.com

Telephone: 631-240-8800

21. Trademarks and Disclaimers

Linea™ COVID-19 and Applied DNA Sciences® are trademarks owned by Applied DNA Sciences, Inc. All other trademarks that appear in this Instructions for Use are the property of their respective owners.

The Linea™ COVID-19 Assay Kit is only for use under the Food and Drug Administration's Emergency Use Authorization for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnosis tests for detection and/or diagnosis of COVID-19.

The Linea™ COVID-19 Assay Kit is for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests, or by similarly qualified non-U.S. laboratories. The Linea™ COVID-19 Assay Kit shall only by used by clinical laboratory personnel who have been trained on authorized instruments.

Use of Linea™ COVID-19 Assay Kit is subject to Applied DNA Sciences' Terms and Conditions of Use located at www.adnas.com/dxcovid.

Not available in all countries.

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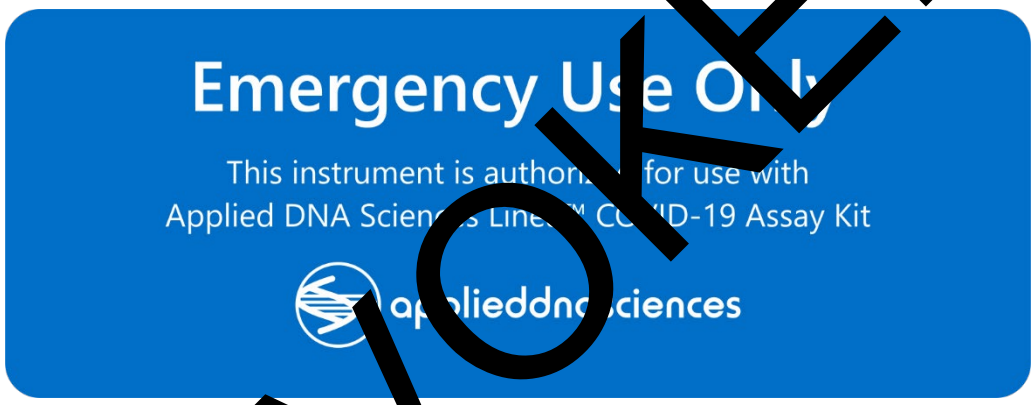
APPENDIX A: ADDITIONAL LABEL

For Qualified QuantStudio 5 Real-Time PCR System

Please print and place this label on the front panel of the instrument. If the instrument includes labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the Linea™ COVID-19 Assay Kit.



REVOKED