united PPE america
Genetics Engineering

INSTRUCTIONS FOR USE

United PPE America CRISPR SARS-CoV-2 Kit

Catalogue No.	**Manufactured under license by**
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Intended Use

The **United PPE America CRISPR SARS-CoV-2 kit** is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The RNA of 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 infective status. Positive results do not rule out bacterial 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 positive 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 **United PPE America CRISPR SARS-CoV-2 kit** is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of non-automated molecular in vitro diagnostic procedures. The **United PPE America CRISPR SARS-CoV-2 kit** is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia caused by a novel coronavirus (SARS-CoV-2) in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. On January 31, 2020, Health and Human Services Secretary Alex M. Azar II declared a public health emergency (PHE) for the United States to aid the nation's healthcare community in responding to SARS-CoV-2. The emergence and rapid spread of SARS-CoV-2 to numerous areas throughout the world, has necessitated preparedness and response in public health laboratories, as well as health care and other areas of society in general. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies and surveillance studies.

Principles of the Procedure

The **United PPE America CRISPR SARS-CoV-2 kit** has been designed to detect fragments of the Open Reading Frame (ORF1ab, "O") gene and the Nucleocapsid ("N") gene of SARS-CoV-2. An included third target is the human RNase P POP7 gene ("RP") which serves as a control for the extraction of the clinical sample in the absence of a positive SARS-CoV-2 result. A dedicated instrument platform (e.g., thermal cycler) is not required. Amplification can be performed using a heat block, and CRISPR complex activation and reporter cleavage can be run in a standard microplate reader capable of fluorescence detection. The entire reaction from RT-LAMP amplification to CRISPR-based detection of the target analytes can be performed in approximately one hour.

The **United PPE America CRISPR SARS-CoV-2 kit** is designed to detect RNA from upper respiratory specimens (such as nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage specimens from patients suspected of COVID-19 by their healthcare provider. RNA is extracted from clinical samples using the PureLink™ Viral RNA/DNA Mini Kit .

The **United PPE America CRISPR SARS-CoV-2 kit** comprises two steps. Step one is a reverse transcriptase loop-mediated amplification (RT-LAMP) where targeted SARS-CoV-2 genomic RNA is reverse transcribed to DNA, and this DNA is amplified by a strand-displacing DNA polymerase. Step two is the transcription of the amplified DNA to activate the collateral cleavage activity of a CRISPR complex programmed to the target RNA sequence. This collateral activity results in cleavage of nucleic acid reporters, resulting in a fluorescent readout detected by a plate reader.

Components and Storage

Materials Required (Provided): Each United PPE America CRISPR SARS-CoV-2 kit consists of the following components:

Item no.	O-Ring Colour on Cap	Assay	Component	Description	Pack Size - 200 tests per kit	Volume per vial
1	Blue	LAMP Assay	2x RT-LAMP Mix	2x Warm Start RT-LAMP Mix	4 vials	1050 µL
2			10x Primers (NO)	N and ORF1ab genes LAMP Primer Mix	2 vials	210 µL
3			10x Primers (RP)	RnaseP POP7 gene LAMP Primer Mix	2 vials	210 µL
4	Green	CRISPR Assay	Cas Mix 1	LwaCas13a Enzyme and T7 RNA Polymerase Mix	2 vials	372 µL
5			Cas Mix 2	Rnase Reporter, rNTP mix, Murine Rnase Inhibitor, MgCl2 and Water Mix	2 vials	783 µL
6	Yellow		crRNA (NO)	Guide crRNA (NO)	2 vials	473 µL
7			crRNA (RP)	Guide crRNA (RP)	2 vials	473 µL

Storage and Handling of Kit Components

- The **United PPE America CRISPR SARS-CoV-2 kit** is shipped on dry ice. The components of the kit should arrive frozen. If one or more of the components are not frozen upon receipt or are compromised during shipment, contact United PPE for assistance.
- Store all components at or below -20°C to prevent degradation of reagents.
- Based on individual component shelf life, the approximate shelf life of the kit is estimated to be 12 months.
- Always check the expiration date prior to use. Do not use expired reagents.
- Always work with **United PPE America CRISPR SARS-CoV-2 kit** components on ice.

Materials Required (Not Provided)

Control Materials

- **Positive Control:** Quantified extracted SARS-CoV-2 genomic RNA (recommended supplier below)
- **Negative Control:** Molecular grade, nuclease-free water

Table 2: Control Materials (not included in this kt)

Control	Supplier	Part Number	Description
SARS-CoV-2 Positive Control (*spc*)	BEI Resources ATCC®	NR-52285 VR1986D™	Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020 or equivalent (BEI NR-52285, or ATCC® VR1986D™), diluted to a concentration of 225 copies/µL
Negative Template Control (*ntc*)	Any supplier	Not Applicable	Nuclease-free water, molecular biology grade

Equipment

Note: Prior to use, ensure that instruments and equipment have been maintained and calibrated according to the manufacturer's recommendations.

Table 4: Equipment

Equipment	Manufacturer	Model
Plate Reader running Gen5 3.08 software	BioTek	NEO2
PC running Microsoft Excel	NA	NA

Table 5: Additional Equipment and Consumables

Equipment and Consumables
0.2 mL strip tubes
1.5 mL snap cap tubes, low bind and nuclease-free
Molecular grade water (nuclease-free)
Mineral oil
Dedicated adjustable P-10, P-20, P-100, P-200, and P-1000 pipettes for sample preparation
Dedicated adjustable P-10 or P-20 for dispensing template RNA
Dedicated adjustable P-10, P-20, P-100, P-200, and P-1000 pipettes for preparing and dispensing master mix
Dedicated adjustable M-10 and M-100 multichannel pipettes for transferring RT-LAMP amplified product and CRISPR Cas detection reaction
Dedicated electronic pipettes for dispensing master mixes (OPTIONAL)
Aerosol barrier tips
384 Corning Black Clear Bottom Low Volume Plate
Plate Optical Seal
Biosafety Cabinet Class II, for the extraction
PCR Workstations, for each portion of the assay set up
Heat block with a heated lid capable of maintaining 61°C or PCR instrument with a heated lid
Vortex
Microcentrifuge
Cold blocks or ice
Tube racks
8 strip tube opener**
Dry Bath/Heat Block*
Tabletop Centrifuge*
Serological Pipette*

*required for RNA Extraction only

**recommended to reduce contamination risk

Table 6: Additional Supplies

Dedicated laboratory coat for each area	Powder-free latex, vinyl or nitrile gloves
Disposable booties	20% (v/v) bleach solution (2.0% w/v sodium hypochlorite in water)
Biohazard bag for tip and tube disposal	70% ethanol

Warnings and Precautions

- For *in vitro* diagnostic use (IVD).
- For use under an Emergency Use Authorization (EUA) only.
- For prescription use only.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC.
- 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 recommendations.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Perform all manipulations of human clinical specimens within a Class II (or higher) biological safety cabinet (BSC). Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
- Report incident to supervisor and consult a physician immediately in the event that infectious materials are ingested or come into contact with mucus membranes, open lacerations, lesions or other breaks in the skin.
- Use of non-recommended reagent volumes may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended volumes and concentrations of the RNA/ DNA sample may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended consumables with instruments may adversely affect test results.
- Do not mix reagents from different lots.
- RNA should be maintained on a cold block or ice during preparation and use to ensure stability.
- Primers, CRISPR guide RNA (crRNA) stocks (including aliquots), enzymes, and RT-LAMP amplification master mix must be thawed and maintained on a cold block at all times during preparation and use.
- Return all components to the appropriate storage condition after preparing the working reagents.
- **Workflow in the laboratory should proceed in a unidirectional manner.**
- Amplification technologies are sensitive to accidental introduction of product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).
    - Maintain separate areas for assay setup and handling of nucleic acids.
    - 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. Proper aseptic technique should always be used when working with nucleic acids.
    - **Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup, handling of extracted nucleic acids, and handling post-amplification products.**
    - **Perform work in a unidirectional workflow in separate locations, from areas**

without specimen/nucleic acid or amplicon to areas with amplified nucleic acid

- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected. **Change gloves after tubes containing amplified product are handled before touching other tubes, equipment, etc.**
- Keep reagent and reaction tubes capped or covered as much as possible.
- Do not interchange vial or bottle caps, as cross-contamination may occur.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 20% bleach and "RNase AWAY®" to minimize risk of nucleic acid or RNase contamination. Residual cleaning solutions should be removed using 70% ethanol.

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

The product contains no substances which at their given concentration, are considered to be hazardous to health or environment.

HMIS

Health	0
Flammability	0
Reactivity	0

Specimen Collection, Handling, and Storage

Adequate, appropriate specimen collection, storage, and transport are important in order to obtain accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results. CLSI MM13-A may be referenced as an appropriate resource.

Collecting the Specimen

- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- A sample collection device is not a part of the assay kit. Follow specimen collection device manufacturer instructions for proper collection methods.
- 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 are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media (i.e. VTM, UTM, M4RT).

Transporting Specimens

- It is the shipper's responsibility to ensure that appropriate shipping materials are used. Please refer to IATA and local regulations.
- Refrigerate specimens at 2-8°C and ship overnight on dry ice.

Storing Specimens

It is recommended that specimens be kept at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C. Repeated freezing and thawing of a specimen should be avoided. If a specimen is kept for retesting, it should be aliquoted in different tubes to avoid freezing and thawing cycles. The temperature in the storage areas should be monitored and recorded regularly to identify potential fluctuations. Domestic refrigerators/ freezers with wide temperature fluctuations are not suitable for the storage of frozen specimens (CDC, 2020).

Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Always include a negative control (*ntc*) for both United PPE America SARS-CoV-2 kit targets (NO, and RP), and a positive control (*spc*) for the SARS-CoV-2 specific target (NO) in each amplification and detection run.
- All clinical samples must be tested for the presence of the human RNaseP (RP) target to

control for specimen quality and extraction, which acts as the internal control.

Nucleic Acid Extraction and Assay Set up

All procedures should be performed in a BSL2 laboratory, and specimens should be handled within a Biological Safety Cabinet. All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross-contamination of samples.

Separate work areas should be used for:

- Nucleic acid extraction
- Reagent preparation (e.g. preparation of master mixes; **NO** amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition area)
- Nucleic acid addition
- RT-LAMP Amplification (e.g. thermocyclers/heat blocks)
- Post-amplification detection (After working in this area, laboratory coat and gloves should be changed and disposed of)

General Handling

- Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination.
- Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed when not in use.
- During the procedure, avoid delays and keep everything on cold blocks or ice when possible to avoid degradation of RNA by endogenous or residual RNases.
- Clean working surfaces, pipettes and equipment with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach.
- Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.

EXPERIMENTAL PROTOCOL:

1. Reagent Controls and Preparation (not included in the kit)

1.1. SARS-CoV-2 Positive Control (*spc*) Preparation:

1.1.1. Precautions: This reagent should be handled with caution to prevent possible contamination. Freeze-thaw cycles should be avoided. Keep cold when thawed.

1.1.2. Dilute the *spc* with nuclease free water to achieve the working concentration of 225 gene copies/µL. Make single use aliquots and store at ≤ -70◦C.

1.1.3. Thaw a single aliquot of the positive control for each experiment and keep on cold block until adding to the RT-LAMP reaction. Discard any unused portion of the aliquot.

1.2. SARS-CoV-2 Negative Control (ntc) Preparation:

1.2.1. Use nuclease-free water for the negative controls.

2. Extraction Methods (not included in the kit)

2.1. Purelink Viral Mini kit (Cat 12280050)

2.1.1. Sample extraction can be performed using the PureLink™ Viral RNA/DNA Mini Kit (Cat. 12280050) as per the manufacturer's instructions with a 200µL samples input volume, 30µL elution volume.

2.2. MagMAX Viral Mini kit (A42352)

2.2.1. Sample extraction can be performed using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (A42352) as per the manufacturer's instructions with a 200µL samples input volume, 50µL elution volume.

2.3. Proteinase K and Heat Treatment (without stabilization solution)

2.3.1. Aliquot 18µL of NP/saline sample into PCR plate or strip tubes

2.3.2. Add 2ul of Proteinase K to each sample.

2.3.3. Heat at 65°C for 6 min, 98°C for 3 min, cool to 4°C prior to use in LAMP reaction.

3. Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP) Assay

3.1. RT-LAMP Master Mix Preparation:

3.1.1. Label a new 1.5 mL microcentrifuge tube for each target ("NO" for SARSCoV-2 or "RP" for extraction control) and prepare a RT-LAMP Master Mix using the recipe in the table 1 blow for extracted RNA samples or table 2 for Proteinase K + heat treated samples.

3.1.2. Pulse vortex RT-LAMP Master Mix for 3 seconds and spin down for 3 seconds in a microcentrifuge after all components are added.

Table 1: Target Specific RT-LAMP Master Mix Recipe for extracted RNA (Viral Extraction Kits)

Reagent	Volume per reaction (µL)	Total Volume (µL)
2x RT-LAMP Mix	10 µL	10 µL x (*N* + 1)
10x Primer (NO or RP)	2 µL	2 µL x (*N* + 1)
Total Volume	12 µL	12 µL x (*N* + 1)

Table 2: Target specific RT-LAMP Master Mix Recipe for treated samples (Proteinase K)

Reagent	Volume per reaction (µL)	Total Volume (µL)
2x RT-LAMP Mix	10 µL	10 µL x (*N* + 1)
10x Primer (NO or RP)	2 µL	2 µL x (*N* + 1)
Water	4 µL	4 µL x (*N* + 1)
Total Volume	16 µL	16 µL x (*N* + 1)

3.1.3. *N* = number of extracted samples plus number of controls. Prepare enough for 1 extra (N + 1) sample to allow for overage during reaction set-up.

3.2. RT-LAMP Amplification in 384 well plate:

3.2.1. Add 12µL (if using Mini Kits) or 16µL (if using Proteinase K) of the RT-LAMP Master Mix into each well for the samples and controls to be amplified.

3.2.2. If using Extraction Kits, add 8µL of the appropriate sample or control to the appropriate wells with LAMP master mix.

3.2.3. If using Proteinase K + heat treated samples add 4µL of sample to the appropriate wells with LAMP master mix.

3.2.4. Add 20µL of mineral oil (not included in the kit) to each of the wells. Note: Add the mineral oil on the side of the well.

3.2.5. Seal the plate with adhesive sealing film.

3.2.6. Incubate the plate on the plate heater set to 61°C for;

30 minutes (Purelink Viral Mini kit (Cat 12280050) or MagMAX Viral Mini kit (A42352)

or

40 minutes (Proteinase K and Heat Treatment (without stabilization solution).

3.2.7. Cool to 21°C.

Table 3: RT-LAMP Assay components and reaction volumes

Reagent	Volume per reaction (µL)
RT-LAMP Master Mix	12 µL / 16 µL
Extraction Kits or Controls / Proteinase K samples	8 µL / 4 µL
Mineral Oil	20 µL
Total volume per well	40 µL

4. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Assay

4.1. CRISPR Cas Master Mix Preparation

4.1.1. Label new 1.5mL tubes with the target name CNO or CRP. Prepare CRISPR Cas Reaction Mix by combining appropriate volumes of Cas Master Mix 1, 2 and guide crRNA according to table 4 below. Once Master Mix 1 and 2 are combined, the mix should be used within 10 minutes.

Table 4: Target CRISPR Cas Master Mix

Reagent	Volume per reaction (µL)	Total Volume (µL)
Cas Mix 1	1.77 µL	1.77 µL x (*N* + 1)
Cas Mix 2	3.73 µL	3.73 µL x (*N* + 1)
Guide crRNA (NO or RP)	4.5 µL	4.5 µL x (*N* + 1)
Total Volume	10 µL	10 µL x (*N* + 1)

4.1.2. Prepare enough for 1 extra (N + 1) sample to allow for overage during reaction set-up.

4.1.3. Pulse vortex for 3 seconds and spin down for 3 seconds in a microcentrifuge after all components are added.

4.1.4. Aliquot Cas Master mix into labelled PCR strip tubes to facilitate addition to the 384 well plate.

4.2. CRISPR Cas Detection

CAUTION - Perform work in a unidirectional workflow in separate locations, from areas without specimen/nucleic acid or amplicon to areas with amplified nucleic acid.

4.2.1. Preheat plate reader to 37°C.

4.2.2. Remove the 384 well plate with the RT LAMP amplification reactions from the plate heater.

4.2.3. Carefully remove the thermal seal from the 384 well plate. Change gloves.

4.2.4. Using a multichannel pipette and fresh tips for each transfer, transfer 10µl of the CRISPR Cas mix into the appropriate wells of the plate (based on plate layout - Cas mix added to each RT-LAMP amplification well).

CAUTION - Do not go to the second stop of the pipette to avoid the introduction of bubbles to the reaction wells.

4.2.5. Seal the 384 well plate with optical seal.

4.2.6. Open the plate reader software to create a read procedure with the following settings:

4.2.6.1. Set temperature to 37°C.

4.2.6.2. Select “Kinetic” run reading with a total read time of 10 minutes with data collection points at 0min, 5min and 10min.

4.2.6.3. Select filter settings in read details to 485nm/528nm filter set with the gains setting set to “extended”.

4.2.6.4. Highlight the appropriate rows and columns based on plate template or read full plate in the plate settings.

4.2.6.5. Press green arrow to start, (i.e., “Create experiment and read now”).

5. Data Analysis and Results Interpretation

After the completion of the plate reader run, select the wells that contain the samples. Use the plate reader software or export the data from these wells to an excel sheet for data analysis.

5.1. Assay Controls

5.1.1. **Negative template control (*ntc*)** reactions are used to monitor reagent and/or environmental contamination. There are two negative control reactions, one for each primer/crRNA set – (i) SARS-CoV-2 (ii) human RNaseP gene. Negative template control reactions are created by substituting the volume of sample material in the RT-LAMP reaction with an equal volume of nuclease-free water.

5.1.2. **Positive control (*spc*)** reactions are used to monitor gross reagent failure, such as reagent degradation, or incorrect assay set-up. There is one positive control reaction, one for SARSCoV-2. Positive control reactions are created by substituting the volume of sample material in the RT-LAMP reaction with an equal volume of extracted SARS-CoV-2 viral RNA at a stock concentration of 225 copies/µL.

5.1.3. For the negative template control (*ntc*), SARS-CoV-2 Positive Controls (*spc*) calculate the ratios as in Table 5 below.

Table 5: Ratio calculations for controls

Sample Type	Reaction Type	Reaction Name	Ratio Calculation	Ratio Calculation Result Interpretation	
				VALID	INVALID
Negative Template Control (*ntc*)	SARS-CoV-2 (N and O gene targets)	NO^{ntc}	$NO^{ntc}(t=10) / NO^{ntc}(t=0)$	< 3	≥ 3
	Human RNaseP gene target	RP^{ntc}	$RP^{ntc}(t=10) / RP^{ntc}(t=0)$		
SARS-CoV-2 Positive Control (*spc*)	SARS-CoV-2 (N and O gene targets)	NO^{spc}	$NO^{spc}(t=10) / NO^{ntc}(t=10)$	≥ 5	< 5
t	= reaction time on fluorescence plate reader (minutes)				
NO^{ntc}	= SARS-CoV2 N and O target negative template control reaction fluorescence				
RP^{ntc}	= RNaseP target negative template control reaction fluorescence				
NO^{spc}	= SARS-CoV2 N and O target positive template control reaction fluorescence				
NO	= SARS-CoV2 N and O gene target reaction fluorescence				
RP	= RNaseP target reaction				

Table 6: Sample Reaction Results Interpretation

Reaction target gene and ratio calculation result		Result Interpretation and Action
SARS-CoV-2 NO NO (t=10) / NO^{ntc} (t=10)	**Human RNaseP** RP (t=10) / RP^{ntc} (t=10)	
≥ 5 (Detected)	N/A	**POSITIVE** for SARS-CoV-2 ACTION: Report Result
< 5 (Not Detected)	≥ 5 (Detected)	**NEGATIVE** for SARS-CoV-2 ACTION: Report Result
< 5 (Not Detected)	< 5 (Not Detected)	INVALID ACTION: See Troubleshooting

Troubleshooting

User Errors

- Good Clinical Laboratory Practices (GCLP) for Molecular Biology Based Tests Used In Diagnostic Laboratories (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel. The user needs to have molecular biology experience and be familiar with the proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection.
- Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment must have calibration up to date for the pipettes and thermocyclers, when applicable.
- A 90 minutes online training for Good Laboratory Practices for Molecular Genetics Testing (Centers for Disease Control and Prevention, 2017) is available at the CDC website at the following link:

https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html

Invalid Results

SARS-CoV-2 Positive Control (*spc*) not detected.

Possible causes:

- ☐ Pipetting errors (control in wrong well, missing a well, inadequate amount of a reagent)
- ☐ Incorrect dilution of positive control nucleic acid
- ☐ Incorrect placement of tubes into heat block/PCR machine
- ☐ Incorrect placement of plate in plate reader
- ☐ Degraded reagents due to incorrect storage temperature
- ☐ Use of expired reagents
- ☐ Use of incorrect reagent

- If the SARS-CoV-2 Positive Control is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction and use a fresh aliquot of the diluted SARS-CoV-2 Positive Control.

- If the positive control fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the samples may need to be re-run.
- If failure of the positive control happens a third time after re-extraction and re-amplification, test with a new lot of **United PPE America CRISPR SARS-CoV-2 kit reagents**.
- If still failing, please contact **United PPE America**.

No Template Control (*ntc*) detection of a SARS-CoV-2 target.

Possible causes:

- ☐ Contamination of one or more reagents during set-up
- ☐ Pipetting errors (control in wrong well, missing a well, inadequate amount of a reagent)
- ☐ Cross contamination of sample wells during RT-LAMP
- ☐ Cross contamination of sample wells during Cas Detection
- ☐ Incorrect placement of tubes into heat block/PCR machine
- ☐ Incorrect placement of plate in plate reader
- ☐ Use of incorrect reagent

- If the *ntc* is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction.
- If the *ntc* fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the samples may need to be re-run.
- If failure of the *ntc* happens a third time after re-extraction and re-amplification, test with a new lot of **United PPE America CRISPR SARS-CoV-2 kit reagents** and a new lot of nuclease free water.
- If still failing, please contact **United PPE America**.

RNaseP (RP) not being detected in patient samples.

Possible causes:

- ☐ Not enough nuclear material in the patient sample
- ☐ The extraction was performed incorrectly
- ☐ Inhibitors present in patient sample
- ☐ Pipetting errors (control in wrong well, missing a well, inadequate amount of a reagent)
- ☐ Incorrect placement of tubes into heat block/PCR machine
- ☐ Incorrect placement of plate in plate reader
- ☐ Use of incorrect reagent

- If the *rp* is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction.
- If the *rp* fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the samples may need to be re-run.
- If failure of the *rp* happens a third time after re-extraction and re-amplification, test with a new lot of **United PPE America CRISPR SARS-CoV-2 kit reagents**.
- If still failing, please contact **United PPE America**.

Limitations

- All users should be qualified by training or experience to perform molecular diagnostic test procedures.
- **United PPE America**, will limit the distribution of this device to laboratories that have been certified to perform CLIA high complexity testing.
- The test was validated for use only with upper respiratory specimens.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- 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.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- If the virus mutates in the RT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- Test performance can be affected because the epidemiology and pathology of disease caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV- 2 is the causative agent for clinical symptoms.
    - The performance of this test has not been established for monitoring treatment of SARS- CoV-2 infection.
    - The performance of this test has not been established for screening of blood or blood product for the presence of SARS-CoV-2.
    - This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

The **United PPE America CRISPR SARS-CoV-2 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/MedicalDevices/Safety/EmergencySituations/ucm161496.htm

Use of the **United PPE America CRISPR SARS-CoV-2 kit** must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization (EUA). To assist clinical laboratories running the **United PPE America CRISPR SARS-CoV-2 kit**, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] will include with reports of the results of the **United PPE America CRISPR SARS-CoV-2 kit**, the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will perform the **United PPE America CRISPR SARS-CoV-2 kit** as outlined in the **United PPE America CRISPR SARS-CoV-2 kit** Instructions for Use Package Insert. Deviations from the authorized procedures, including authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the **United PPE America CRISPR SARS-CoV-2 kit** are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will 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 to United PPE America any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained/experienced in molecular in vitro diagnostic test techniques, use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- United PPE America and authorized laboratories will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

Performance Characteristics

Analytical Sensitivity (Limit of Detection):

The Limit of Detection (LoD) for the United PPE America CRISPR SARS-CoV-2 kit was determined to be the lowest concentration of Zeptometrix NATtrol SARS-CoV-2 viral particles at which ≥ 95% of all replicates test positive.

To determine the LoD, limiting dilutions of Zeptometrix NATtrol SARS-CoV-2 viral particles were spiked into negative NP matrix and purified using the MagMAX Viral RNA isolation kit with 30 minute LAMP amplification. After extraction, the samples were processed according to the United PPE America SARS-CoV-2 kit workflow. Initially three (3) replicates of 5 dilutions were tested. The LoD was confirmed by testing twenty (20) replicates at 1cp/µL and 0.5cp/µL in the same sample matrix as above. Results are shown in Tables 14 and 15 below

Table 14: LoD determination MagMAX Viral RNA Kit

Viral Particles in Sample (cp/µL NP matrix)	Total Positive (NO)	Total Positive (RNaseP)
100	3/3	3/3
10	3/3	3/3
1	1/3	3/3
0.01	0/3	3/3
0	0/3	3/3
Positive Control	3/3	0/3
Negative Control	0/3	0/3

The LoD was confirmed by testing at 1cp/µL and 0.5cp/ µL . The LoD of each target was determined to be the concentration at which ≥19/20 replicates for each assay target was positive. The LoD for NO target was determined to be 1 cp/µL NP.

Table 15: LoD confirmation MagMAX Viral RNA Kit

Target	Viral Particles in Sample (cp/ µL NP)	# of Samples	# of Detected	Detection Rate (%)
NO	1	20	19	95
NO	0.5	20	15	75

The confirmed LoD for the United PPE America CRISPR SARS-CoV-2 kit is 1 cp/uL NP matrix

To determine the LoD of the Proteinase K and Heat treatment method, limiting dilutions of NATtrol SARS-CoV-2 viral particles were spiked into 0.9% saline. The samples were processed according to the United PPE America ™ SARS-CoV-2 kit workflow. Initially three (3) replicates of 5 dilutions were tested. The LoD was confirmed by testing twenty (20) replicates at 1cp/µL and 0.5cp/µL in the same sample matrix as above. Results are shown in Tables 16 and 17 below.

Table 16: LoD determination for Proteinase K and Heat with Saline

Viral Particles in Sample (cp/µL in Saline)	Total Positive (NO)
100	3/3
10	3/3
1	0/3
0.1	0/3
0.01	0/3
0	0/3

The LoD was confirmed by testing at 10cp/µL and 5cp/ µL. The LoD of each target was determined to be the concentration at which ≥19/20 replicates for each assay target were positive. The LoD for NO target was determined to be 10 cp/µL saline.

Table 17: LoD confirmation for Proteinase K and Heat with Saline

Target	Viral Particles in Sample (cp/ µL NP)	# of Samples	# of Detected	Detection Rate (%)
NO	10	20	19	95
NO	5	20	16	80

Analytical Inclusivity:

In silico analysis was performed to determine reactivity of the **UNITED PPE AMERICA CRISPR SARS-CoV-2 kit**. Analysis was carried out to determine the *in silico* inclusivity against all available Sars-Cov2 genomes, including 4 emerging variants. First, N = 80019 Sars-Cov2 viral genomes annotated as complete up to a collection date of 4/5/21 were curated from NCBI and GISAID. Of these, N = 1486 viral genomes were removed due to having more than 1% of the genome consisting of ambiguous bases. A Multi-sequence alignment (MSA) was constructed using all remaining N = 78533 Sars-Cov2 viral genomes, and all primers and crRNAs were subsequently aligned to the MSA in order to assess homology. 98.5% (77326 genomes) SARS-CoV-2 genomes had 100% homology to all primer and guides

sequences for either the ORF1ab or N targets and therefore will be detected by at least one of the **UNITED PPE AMERICA CRISPR SARS-CoV-2 kit** target analyte tests.

Finally, in silico inclusivity was also evaluated against the emerging variants. To that end, N = 992 B.1.1.7 variants, N = 577 B.1.351 variants, N = 78 P1 variants, and N = 1429 B.1.429 variants were analyzed as above. All genomes (N = 3076) had 100% homology to all primer and crRNA binding regions for either the ORF1ab or N targets. It is therefore determined that these variants will be detected by at least one of the **UNITED PPE AMERICA CRISPR SARS-CoV-2 kit** target analyte tests.

Analytical Specificity:

All primers and crRNAs were analyzed with BLASTn using public domain nucleotide databases and excluding results with the taxonomy ID for SARS-CoV-2. The nt database was used as described above for general exclusivity testing except this database was also filtered to only include sequences for taxonomy identifiers (taxid) of the high priority organisms (see Table 15 below). Search parameters were automatically adjusted for short input sequences. Additional settings included: expect threshold 1000, match score 1 and mismatch scores -3, and penalty to create gap in an alignment 5 and extend a gap in an alignment 2.

Significant homology (>80%) was identified between Bat SARS and a single primer in the ORF1ab target. Significant homology (>80%) was identified between Bat SARS, SARS and Influenza B and a single primer or guide in the N target. Since LAMP amplicon generation is not possible with a single primer there is little risk of cross reactivity with the organisms.

Of the organisms identified with high homology, microbial interference is unlikely from Bat SARS or SARS because they are not common human pathogens. Microbial interference from Influenza B is unlikely because there are 2 mismatches at the terminal 3' end of the primer.

In summary, the ORF1ab and N primers and crRNAs designed for the specific detection of SARS-CoV-2 showed no significant homologies that are likely to cause cross-reactivity or microbial interference with the human genome and transcriptome or with other organisms including bacteria and viruses.

Table 18 – List of High Priority Organisms for Specificity / Exclusivity Assessment

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae

	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Human genome
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

Wet testing against high risk pathogenic organisms of the respiratory tract, selected based on disease prevalence, disease risk, homology to assay specific targets and homology to the SARS-CoV-2 genome, was performed to confirm the results of the *in silico* analysis. Each organism identified in **Table 19** below was tested in triplicate with the United PPE America CRISPR SARS-CoV-2 kit by spiking into the amplification reaction followed by detection. All replicates were negative for SARS-CoV-2 detection.

Table 19. Wet testing of potential cross-reactive organisms

Organism	ATCC Cat. Number	Concentration	NO	RNaseP
Human coronavirus 229E	ATCC® VR-740D	6.67 x 10^5 copies/mL	0/3	3/3
Human coronavirus OC43	ATCC® VR-1558D	6.67 x 10^5 copies/mL	0/3	3/3
Human coronavirus HKU1	ATCC® VR-3262SD	6.67 x 10^5 copies/mL	0/3	3/3
Human coronavirus NL63	ATCC® 3263SD	6.67 x 10^5 copies/mL	0/3	3/3
Influenza A	VR-95DQ	6.67 x 10^5 copies/mL	0/3	3/3
Influenza B	VR-1885DQ	6.67 x 10^5 copies/mL	0/3	3/3
Respiratory syncytial virus	ATCC® VR-1580DQ	6.67 x 10^5 copies/mL	0/3	3/3
Pseudomonas aeruginosa	ATCC® 27853D-5	6.67 x 10^6 copies/mL	0/3	3/3
Staphylococcus epidermis	ATCC® 12228D-5	6.67 x 10^6 copies/mL	0/3	3/3
Candida albicans	ATCC® 10231D-5	6.67 x 10^6 copies/mL	0/3	3/3

Endogenous Interference Substances Studies:

The **United PPE America CRISPR SARS-CoV-2 kit** uses a conventional nucleic acid extraction method; we do not anticipate interference from common endogenous substances using this method.

Clinical Evaluation:

The clinical evaluation was performed on N=60 individual remnant nasopharyngeal swab samples confirmed to be positive (30 samples) or negative (30 samples) for SARS-CoV-2 by RT-qPCR. The samples were randomized, then processed using the **United PPE America CRISPR SARS-CoV-2 kit** workflow with MagMAX viral RNA extraction. The results, as presented in Table 20 below, showed 100% agreement with the expected results for both the positive and negative specimens.

Table 20: Clinical Evaluation MagMAX Viral RNA Kit

		RT-qPCR Assay Results	
		+	-
MagMax Assay Result	+	30	0
	-	0	30

Positive percent agreement (Sensitivity) = 100% (95% CI: 90.5-99.9%)

Negative percent agreement (Specificity) = 100% (95% CI: 90.5-100%)

A second clinical evaluation was performed on N=45 individual remnant nasopharyngeal swab samples in saline confirmed to be positive (20 samples) or negative (25 samples) for SARS-CoV-2 by RT-qPCR. The samples were randomized, then processed using the **United PPE America CRISPR SARS-CoV-2 kit** workflow with Proteinase K and heat sample treatment. The results, as presented in Table 21 below, showed 100% agreement with the expected results for both the positive and negative specimens.

Table 21: Clinical Evaluation Proteinase K and Heat with NP/Saline

		RT-qPCR Assay Results	
		+	-
Proteinase K and Heat Assay Result	+	20	0
	-	0	25

Positive percent agreement (Sensitivity) = 100% (95% CI: 86.1-99.9%)

Negative percent agreement (Specificity) = 100% (95% CI: 88.7-100%)

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

The product contains no substances which at their given concentration, are considered to be hazardous to health.

HMIS

Health	0
Flammability	0
Reactivity	0

Symbols Used In Packaging

Symbol	Definition
CE	CE Marking
	Manufactured By
mm-yyyy	Date Format (month-year)

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CDC guidelines for Sample collection –
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

FDA EUA guidance –
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-test s-coronavirus-disease-2019-during-public-health-emergency

Thermo Fisher viral RNA extraction kit PureLink™ Viral RNA/DNA Mini Kit (Cat# 12280050) Kit for RNA isolation
https://www.thermofisher.com/order/catalog/product/12280050#/12280050 Catalog Number 12280050 Publication NumberMAN0000562

Contact Information, Ordering, and Product Support

Information and product support can be obtained from:

Contact: United PPE America Customer Support
Email: support@unitedppeamerica.com

Phone: +1 917-397-6807
Website: www.unitedppeamerica.com

Product support information

- Product FAQs
- Technical support
- Order and web support

Product documentation

- User guides, manuals, and protocols
- Fact Sheet for Healthcare Providers
- Fact Sheet for Patients
- Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.