BD SARS-CoV-2 Reagents for BD MAX™ System

For Emergency Use Authorization (EUA) only
For *In Vitro* Diagnostic Use
For use with the BD MAX™ System

REF 445003-01
P0252(07)
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English

2 °C 25 °C IVD Rx Only bd.com/e-labeling

INTENDED USE

The BD SARS-CoV-2 Reagents for BD MAX™ System is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, anterior nasal, mid-turbinate, and oropharyngeal swab specimens, nasopharyngeal wash/aspirate or nasal aspirates obtained from individuals suspected of COVID-19 by their healthcare provider or from individuals without symptoms or other epidemiological 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 moderate or high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory samples 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 BD SARS-CoV-2 Reagents for BD MAX™ System is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR, *in vitro* diagnostic procedures, and use of the BD MAX™ System. The BD SARS-CoV-2 Reagents for BD MAX™ System is only for use under the Food and Drug Administration's Emergency Use Authorization.

EXPLANATION OF THE TEST

Total nucleic acid (TNA) is isolated and purified using BD SARS-CoV-2 Reagents for BD MAX™ System from anterior nasal, mid-turbinate, nasopharyngeal, or oropharyngeal swabs collected in BD Universal Viral Transport System (UVT) or Copan Universal Transport Media System (UTM) and anterior nasal swabs collected in 0.85% saline. Patient sample is transferred to the Sample Buffer Tube provided with the BD SARS-CoV-2 Reagents for BD MAX™ System and placed in the BD MAX™ System.
The BD MAX™ ExK™ TNA-3 unitized reagent strip contains a combination of lytic and extraction reagents designed to perform cell lysis and TNA extraction. Eluted TNA is transferred to SARS-CoV-2 primers and probes and to the BD MAX™ TNA MMK master mix. The final rehydrated master mix is transferred to a PCR cartridge for rRT-PCR.

The BD SARS-CoV-2 Reagents for BD MAX™ System utilizes multiplexed primers and probes targeting RNA from the nucleocapsid phosphoprotein gene (N1 and N2 regions) of the SARS-CoV-2 coronavirus, and the human RNase P gene. The primer and probe sets are based on the United States Centers for Disease Control and Prevention (US CDC) assay for specific detection of SARS-CoV-2 by amplifying two unique regions of the N gene (i.e., N1 and N2).

An internal control targeting the human RNase P gene will be co-amplified along with N1 and N2 gene targets (if present) and will serve as an endogenous nucleic acid extraction control present in all properly collected patient samples. This control serves as both an extraction control and an internal amplification control.

PRINCIPLES OF THE PROCEDURE

A combination of lytic and extraction reagents is used to perform cell lysis and DNA/RNA extraction. Nucleic acids released from the target organisms are captured on magnetic affinity beads. The beads, together with the bound nucleic acids, are washed and the nucleic acids are eluted by a combination of heat and pH variation. Neutralization buffer is used to rehydrate BD SARS-CoV-2 Reagents for BD MAX™ System Primers and Probes. Eluted TNA is added to the rehydrated primers and probes, mixed, and transferred to BD MAX™ TNA MMK master mix for rehydration. After reconstitution, the BD MAX™ System dispenses a fixed volume of RT-PCR-ready solution containing extracted nucleic acids into the PCR Cartridge. Microvalves on the cartridge are sealed by the system prior to initiating PCR in order to contain the amplification mixture and thus prevent evaporation and contamination.

The amplified cDNA targets are detected using hydrolysis (TaqMan®) probes, labeled at one end with a fluorescent reporter dye (fluorophore), and at the other end, with a quencher moiety. Probes labeled with different fluorophores are used to detect the target analytes in different optical channels of the BD MAX™ System. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of target cDNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'–3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the cDNA template. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the optical channels is directly proportional to the quantity of the corresponding probe that is hydrolyzed. The BD MAX™ System monitors these signals at each cycle of the PCR and interprets the data at the end of the reaction to provide qualitative test results for each analyte.

REAGENTS AND MATERIALS

REF	Contents	Quantity
445003-01	**BD MAX™ ExK™ TNA-3 Sample Buffer Tube** (with 25 septum caps)	24
	BD MAX™ TNA Unitized Reagent Strip (TNA) Unitized Reagent Strip containing all liquid reagents and disposable pipette tips necessary for specimen processing and TNA extraction.	
	BD MAX™ ExK™ TNA Extraction Tube (B4) Dried extraction reagent containing magnetic affinity beads and Proteinase K.	24 (2 x 12 tubes)
	BD SARS-CoV-2 Reagents for BD MAX™ System Primers and Probes Dried primers and probes for SARS-CoV-2	
	BD MAX™ TNA MMK (C3) Dried PCR Master Mix containing dNTPs and RT-polymerase	

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX™ System (BD Catalog No. 441916)
- BD MAX™ Sample Rack (BD Catalog No. 441935, 443550, 443551, 444807, or 444808)
- BD MAX™ PCR Cartridges (BD Catalog No. 437519)
- SARS CoV-2 and RNase P Controls
- Copan UTM Collection Kit
- BD UVT Collection Kit
- 0.85% Saline
- Vortex Genie 2 (VWR Catalog No. 58815-235 or equivalent)
- Multi-Tube Vortex Mixer (VWR Catalog No. 58816-115 or equivalent)
- Rack compatible with a multi-tube vortexer (e.g., Cryogenic Vial Holder or equivalent)
- Variable Volume Calibrated Pipettor (750 µL volume capable)
- Aerosol resistant micropipette tips
- Disposable gloves, powderless

WARNINGS AND PRECAUTIONS

Danger

H311 Toxic in contact with skin.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
H350 May cause cancer.
H360 May damage fertility or the unborn child.
H411 Toxic to aquatic life with long lasting effects.
P201 Obtain special instructions before use.
P202 Do not handle until all safety precautions have been read and understood.
P233 Keep container tightly closed.
P261 Avoid breathing dust/fume/gas/mist/vapors/spray.
P264 Wash thoroughly after handling.
P271 Use only outdoors or in a well-ventilated area.
P272 Contaminated work clothing should not be allowed out of the work place.
P273 Avoid release to the environment.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P281 Use personal protective equipment as required.
P284 [In case of inadequate ventilation] wear respiratory protection.
P308+P313 IF exposed or concerned: Get medical advice/attention.
P332+P313 IF skin irritation occurs: Get medical advice/attention.
P333+P313 IF skin irritation or rash occurs: Get medical advice/attention.
P337+P313 IF eye irritation persists: Get medical advice/attention.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P302+P352 IF ON SKIN: Wash with plenty of water.
P312 Call a POISON CENTER/doctor if you feel unwell.
P321 Specific treatment.
P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor/...
P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P361+P362 Take off contaminated clothing.
P363 Wash contaminated clothing before reuse.
P391 Collect spillage.
P403 Store in a well-ventilated place.
P405 Store locked up.
P501 Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

- For *in vitro* diagnostic use under 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.
- This product has been authorized for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform moderate or 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 for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C.§360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in the CLSI Document M29-A4[1] and in Biosafety in Microbiological and Biomedical Laboratories.[2] Only personnel proficient in handling infectious materials and the use of BD SARS-CoV-2 and BD MAX™ System should perform this procedure.

- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, follow appropriate site procedures.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Protect reagents against heat and humidity. Prolonged exposure to humidity may affect product performance.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or re-use caps, as contamination may occur and compromise test results.
- Check Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Check Unitized Reagent Strips to ensure that all pipette tips are present.
- Proceed with caution when using chemical solutions, as Extraction Tube barcode readability may be altered.
- Good laboratory technique is essential to the proper performance of this assay. Extreme care should be taken to preserve the purity of all materials and reagents.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the BD SARS-CoV-2 components, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges.
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridges are designed to prevent contamination.
- The laboratory should routinely perform environmental monitoring to minimize the risk of cross-contamination.
- Wear protective clothing and disposable gloves while handling all reagents.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth.
- Do not smoke, drink, chew or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with local, state, provincial and/or federal regulations.
- Consult the BD MAX™ System User's Manual[3] for additional warnings, precautions and procedures.

STORAGE

- BD SARS-CoV-2 Reagents for BD MAX™ System kit ships at ambient temperature and is stable at 2–25 °C through the stated expiration date. Do not use if expired.
- The BD MAX™ TNA Extraction Tubes (B4), BD MAX™ TNA MMK (C3), and BD SARS-CoV-2 Reagents for BD MAX™ System Primers and Probes are provided in sealed pouches. To protect from humidity, immediately re-seal after opening.

INSTRUCTIONS FOR USE

Swab Specimen Collection/Transport in Universal Viral Transport (UVT) or Universal Transport Medium (UTM)

Note: Wear gloves when handling specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.

1. Nasal, nasopharyngeal, or oropharyngeal swab specimens should be collected and expressed directly into the BD Universal Viral Transport System or the Copan Universal Transport Media System according to their respective package insert instructions.
2. After collection, transport the UVT/UTM specimen according to the manufacturer's instructions for use.
3. If a delay in testing or shipping is expected, store specimens at -70 °C or below. Frozen storage for up to 30 days was evaluated. Frozen specimens should not exceed two (2) freeze thaw cycles.

Swab Specimen Collection/Transport in Saline

Note: Wear gloves when handling specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.

1. Nasal swab specimens should be collected and expressed directly into the saline tube.
2. Store specimens at 2–8 °C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70 °C or below.

Note: Sample stability when using BD SARS-CoV-2 Reagents for BD MAX™ System has not been established for suggested temperatures and time but is based on CDC guidelines.[4]

BD MAX™ Sample Buffer Tube Preparation for use with nasal, nasopharyngeal, or oropharyngeal swab specimens in Universal Viral Transport (UVT) or Universal Transport Media (UTM) or nasal swab specimens in 0.85% saline

Note: Wear gloves when handling specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.

Note: If frozen, allow Universal Viral Transport (UVT) or Universal Transport Media (UTM) specimen to come to room temperature before proceeding.

1. Uncap the BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM or saline specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube.
2. Recap the tube with a blue septum cap and vortex or mix by inversion 5 times.
3. Label the BD MAX™ TNA-3 Sample Buffer Tube with patient information.

   Note: Do not obscure the barcodes on the tube. Obscuring the barcode may result in BD MAX™ System catalog failure and inability to test the sample.
4. Repeat Steps 1 to 3 for each UVT/UTM or saline sample that will be tested on the BD MAX™ System.
5. Proceed directly with the BD MAX™ System Operation.

BD MAX™ System Operation

Note: Refer to the BD MAX™ System User's Manual[3] for detailed instructions (Operation section).

1. Power on the BD MAX™ System (if not already done) and log in by entering **<user name>** and **<password>**.
2. Gloves must be changed before manipulating reagents and cartridges.
3. Remove the required number of TNA Unitized Reagent Strips from the BD SARS-CoV-2 Reagents for BD MAX™ System kit. Gently tap each Unitized Reagent Strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes. Remove the required number of Extraction Tube(s) from the protective pouch. Remove excess air, and close pouches with the zip seal.
4. From the BD SARS-CoV-2 Reagents for BD MAX™ System kit, remove the required number of BD MAX™ TNA MMK Master Mix Tube(s) and BD SARS-CoV-2 Reagents for BD MAX™ System Primers and Probes Tube(s) from their protective pouches. Remove excess air, and close each pouch with the zip seal.
5. For each specimen to be tested, place one (1) Unitized Reagent Strip on the BD MAX™ System Rack. Assemble the strip as in Figure 1:

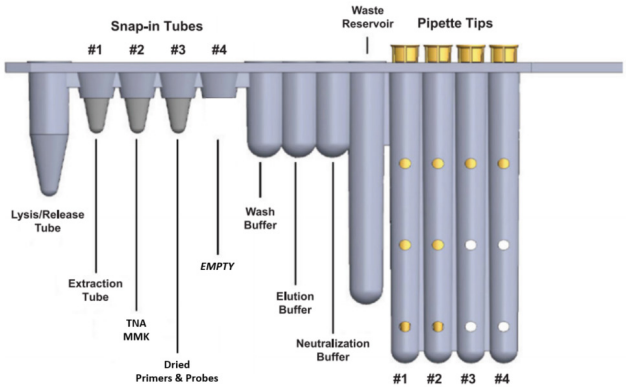


Figure 1: Snap Extraction Tubes and Master Mix Tube into Unitized Reagent Strips

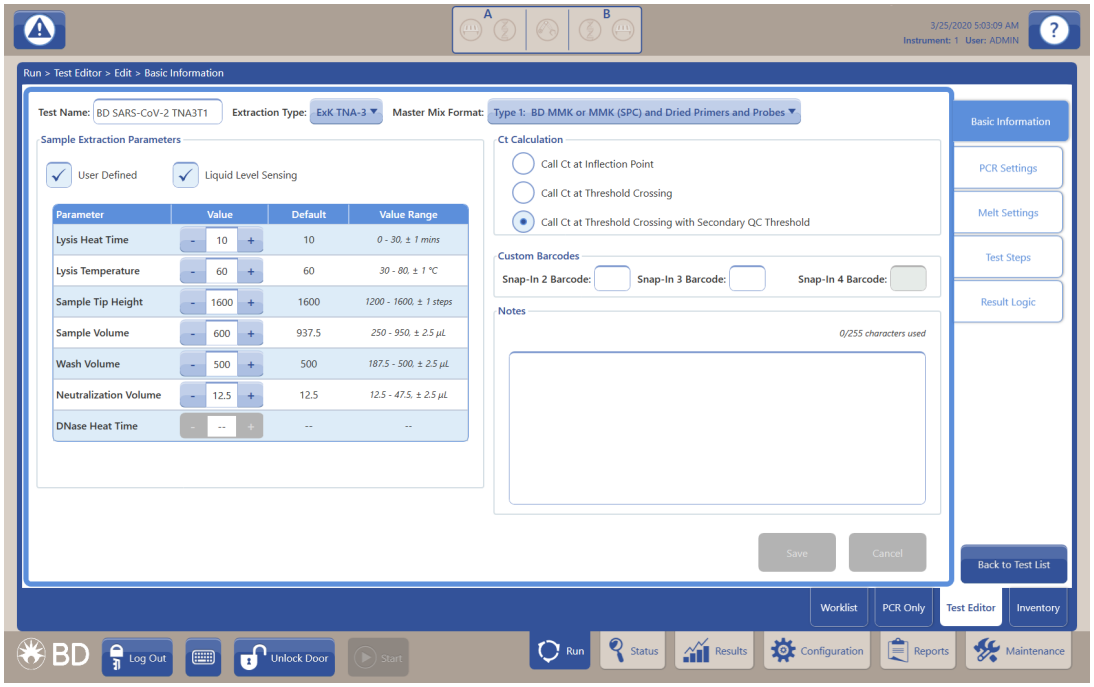
Note: Failure to add extraction tube or master mix tubes may result in instrument contamination.

Note: A conical snap-in tube is fully seated in the strip when a 'click' is heard. Refer to above for reagent placement in the Unitized Reagent Strip.

- Position 1= Snap the BD MAX™ ExK™ TNA-3 Extraction Tube into Position 1.
- Position 2= Snap the BD MAX™ TNA MMK Master Mix Tube into Position 2.
- Position 3= Snap the BD SARS-CoV-2 Reagents for BD MAX™ System Primers and Probes into Position 3.
- Position 4= Leave Position 4 empty (no conical snap-in tube).

6. Create the User Defined Protocol (UDP) as follows:
   - Navigate to Run > Test Editor tab.
   - Click "Create".
   - Complete each section of the user protocol as outlined in the screen shots below.

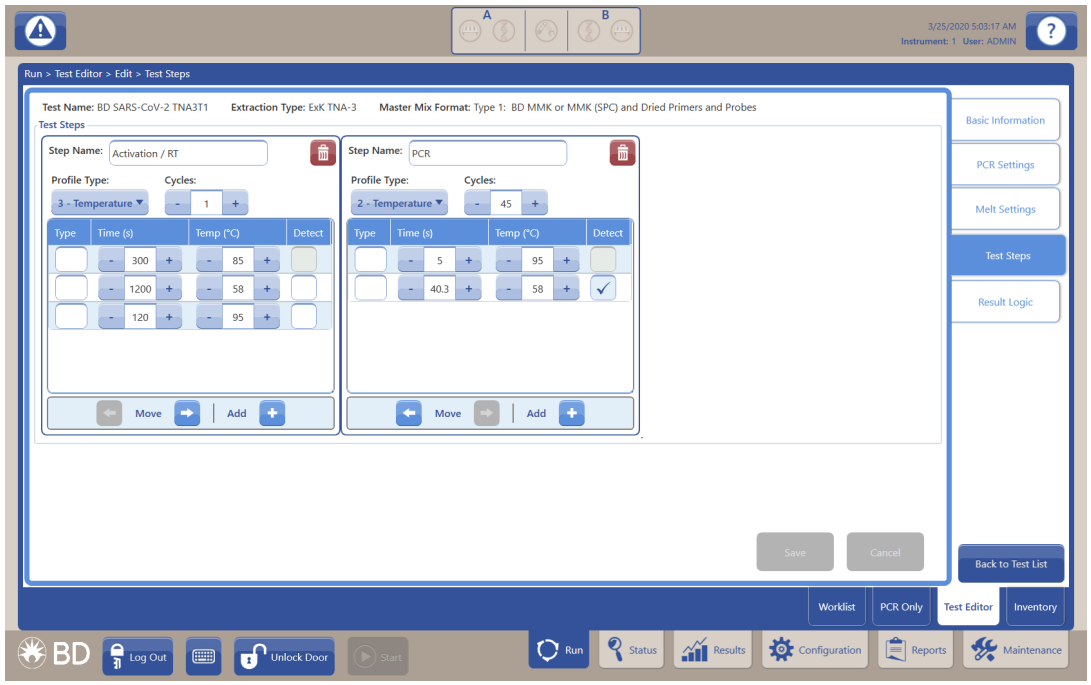
Basic Information Section



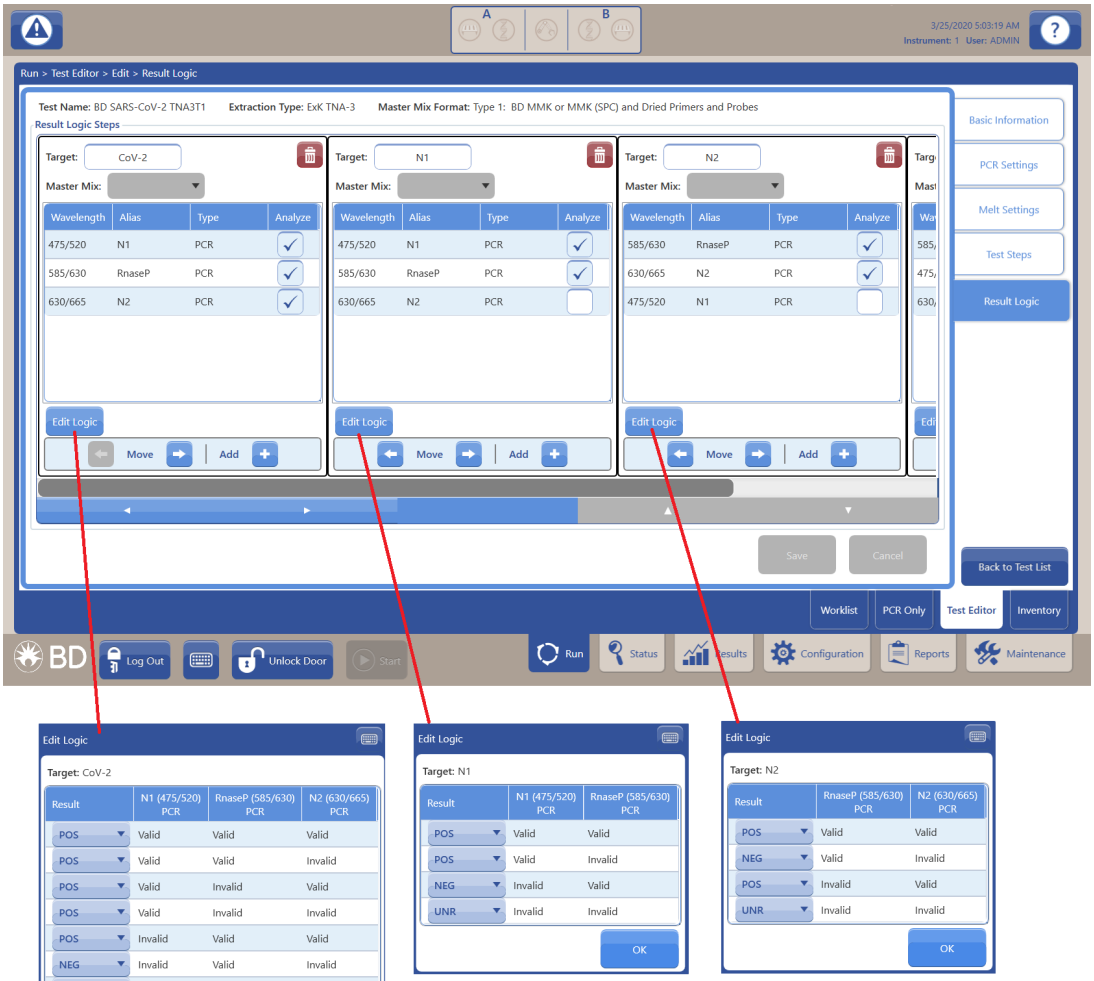
PCR Setting Section

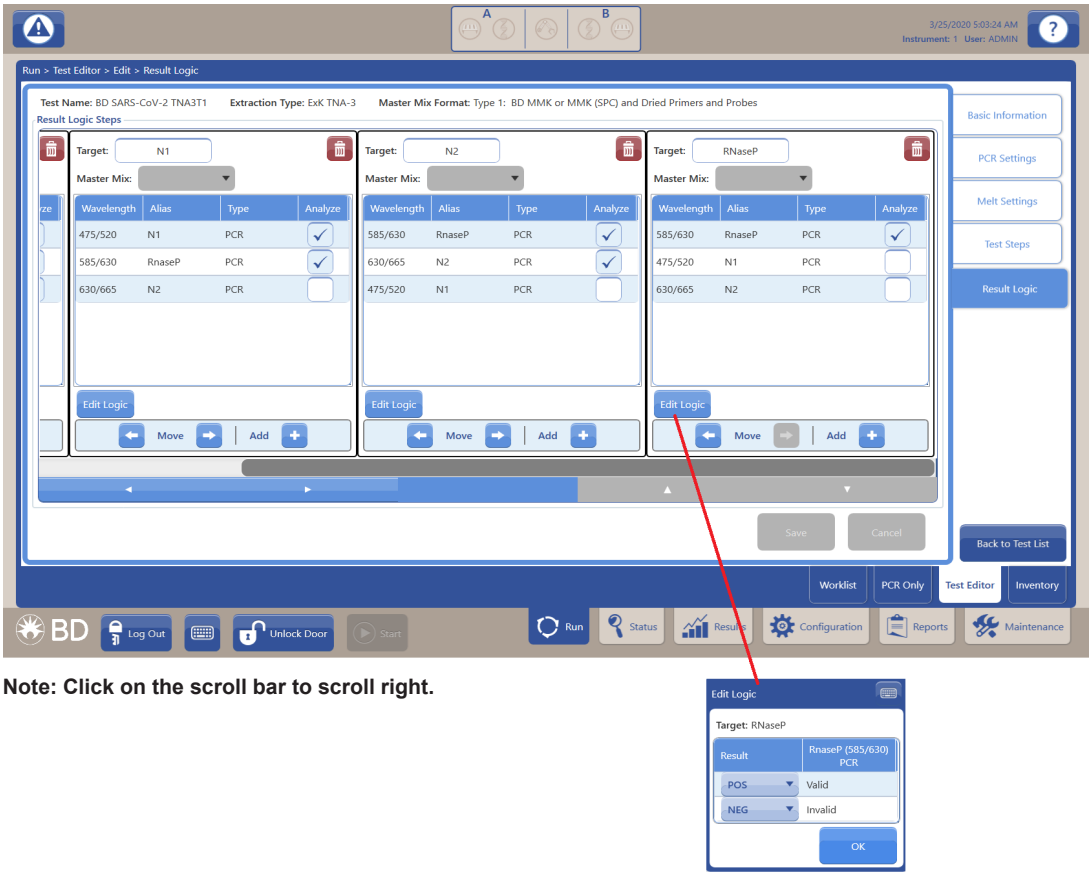
Melt Settings Section

Test Steps Section



Result Logic Section (enter result logic by clicking the “Edit Logic” button)





Note: Click on the scroll bar to scroll right.

7. Click **<SAVE>** after all information has been entered into the Test Editor. The UDP only needs to be created once, and steps 6 and 7 do not need to be repeated for subsequent runs.
8. Click on the Run tab, then Inventory. Enter the kit lot number for the BD SARS-CoV-2 Reagents for BD MAX™ System kit in the barcode field (for lot traceability) by either scanning the barcode with the scanner or by manual entry and then save.

   Note: Repeat step 8 each time a new kit lot is used.
9. Navigate to the Worklist (RUN > WORKLIST). Using the pull down menu select the UDP previously created in Step 6 (example: BD SARS-CoV-2 TNA3).
10. Enter the Sample Buffer Tube ID, Patient ID and Accession Number (if applicable) into the Worklist, either by scanning the barcode with the scanner or by manual entry.
11. Select the appropriate kit lot number (found on the outer box) from the pull down menu.
12. Repeat Steps 9 to 11 for all remaining Sample Buffer Tubes.
13. Place the Sample Buffer Tubes into the BD MAX™ System Rack(s) corresponding to the Unitized Reagent Strips previously assembled.

14. Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (refer to Figure 2).

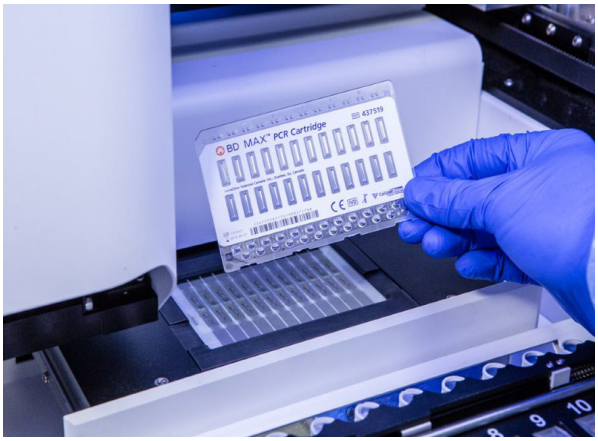


Figure 2: Load BD PCR Cartridges

15. Load rack(s) onto the BD MAX™ System (refer to Figure 3).

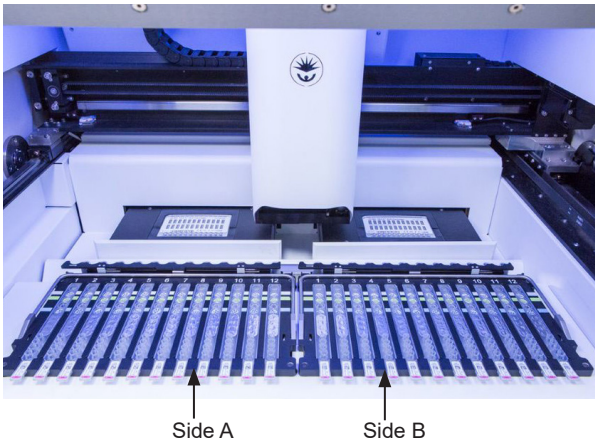


Figure 3: Load Rack(s) onto the BD MAX™ System

16. Close the BD MAX™ System lid and click **<Start>** to begin the processing.

QUALITY CONTROL

Quality control procedures monitor the performance of the assay. Laboratories must establish the number, type, and frequency of testing of control materials according to guidelines or requirements of local, provincial, state, and federal and/or country regulations or accreditation organizations in order to monitor the effectiveness of the entire analytical process. For general Quality Control guidance, the user may wish to refer to CLSI MM3 and EP12.[1,2]

External Control materials are not provided by BD. External Positive and Negative Controls are not used by the BD MAX™ System software for the purpose of sample test result interpretation. External Controls are treated as if they were patient samples. (Refer to the table in the Results Interpretation section for the interpretation of External Control assay results.)

It is recommended that one (1) External Positive Control and one (1) External Negative Control be run at least daily until adequate process validation is achieved on the BD MAX™ System in each laboratory setting. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Reduced frequency of control testing should be in accordance with applicable regulations.

The External Positive Control is intended to monitor for substantial reagent failure. The External Negative Control is intended to detect reagent or environmental contamination (or carry-over) by target nucleic acids.

Various types of External Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program.

External Negative Control: A previously characterized sample known to be negative or a Sample Buffer Tube with RNase P positive control added. BD recommends that the External Negative Control be prepared prior to the External Positive Control in order to reduce the potential for contamination as a result of control preparation.

External Positive Control: Commercially available control material from Microbiologics, BioGX, CDC/IDT, or other authorized control material may be used.

For the preparation of External Control suspensions, it is recommended that RNA suspensions be prepared in the Sample Buffer Tube according to manufacturer's instructions.

All External Controls should yield the expected results (positive for External Positive Control, negative for External Negative Control).

An External Negative Control that yields a positive result is indicative of sample handling and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.

An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the System Error Summary section of the BD MAX™ System User's Manual[3] for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new assay kit.

RESULT INTERPRETATION

Results are available on the results tab in the Results window on the BD MAX™ System monitor. The BD MAX™ System automatically interprets the test result when the SARS-CoV-2 User Defined Protocol (UDP) is used.

External Negative and Positive Controls

If the positive or negative controls are processed in the run and do not exhibit the expected performance as described in the Control Interpretations table below, the assay may have been set up/or executed improperly, or reagent or equipment malfunction could have occurred. In this case, invalidate the run and re-test all samples in that run.

The RNase P gene serves as both a sample extraction control (EC) and an internal amplification control (IAC). In the event that both N1 and N2 region results are negative, an RNase P result must be positive for the BD SARS-CoV-2 result to be a valid negative result. When either N1 or N2 target result is positive, the RNase P result is ignored.

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

Table 1: External Control Expected Results

Control Type		Used to Monitor	Expected Results			
			N1	N2	RNase P	CoV-2
External Negative Control	Known Negative Sample	Reagent and/or environmental contamination and reagent failure including primer and probe integrity	NEG	NEG	POS	NEG
	RNase P Positive Control		NEG	NEG	POS	NEG
External Positive Control	N1 and N2 Positive Control	Substantial reagent failure including primer and probe integrity	POS	POS	N/A	POS

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the external 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.

The table below lists the expected results. If results are obtained that do not follow these guidelines, re-extract and re-test the sample. If repeat testing yields similar results, collect a fresh sample from the patient for testing.

Table 2: Interpretation of Patient Specimen Results

N1 Region	N2 Region	Extraction Control (RNase P)	CoV-2	Result Interpretation[a,b]	Actions
POS	POS	POS/NEG	POS	Positive	Report as Detected
POS	NEG/UNR	POS/NEG	POS	Positive	Report as Detected
NEG/UNR	POS	POS/NEG	POS	Positive	Report as Detected
NEG	NEG	POS	NEG	Negative	Report as Not Detected
UNR	UNR	NEG	UNR	UNR	Repeat Test[c]

[a]UNR = Unresolved

[b]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

[c]Repeat Test by preparing a fresh Sample Buffer Tube from the original primary sample.

UNRESOLVED, INDETERMINATE, AND INCOMPLETE RESULTS

When an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained a repeat test from the primary sample must be performed. If an External Control fails, repeat testing of all specimens conducted on the same day using freshly prepared External Controls (see Quality Control).

Unresolved Result

Unresolved results may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or RNase P amplification. Sample(s) can be repeated from the primary sample. Uncap the BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM/saline specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube. Restart from the BD MAX™ System Operation section.

Indeterminate Result

Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from the primary sample. Uncap the BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM/saline specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube. Restart from the BD MAX™ System Operation section.

Incomplete Result

Incomplete results may be obtained in the event that Specimen Preparation or the PCR did not reach its expected time points. Sample(s) can be repeated from the primary sample. Uncap the BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM/saline specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube. Restart from the BD MAX™ System Operation section.

External Control Failure

External Controls should yield expected results when tested. If samples have to be repeated due to an incorrect External Control result, the samples should be repeated from the primary sample along with freshly prepared External Controls. Restart from the BD MAX™ System Operation section.

LIMITATIONS OF THE PROCEDURE

- BD SARS-CoV-2 Reagents for BD MAX™ System has been evaluated only for use on the BD MAX™ System.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been designed for the detection of SARS-CoV-2 RNA in nasopharyngeal, oropharyngeal, and nasal swab samples collected in BD Universal Viral Transport System (UVT) or Copan Universal Transport Media System (UTM) and nasal swabs collected in 0.85% saline (self-collected under supervision of a healthcare provider or healthcare provider-collected). BD SARS-CoV-2 Reagents for BD MAX™ System performance was evaluated using nasopharyngeal and nasal swabs. Use of BD SARS-CoV-2 Reagents for BD MAX™ System with other sample types, including nasopharyngeal wash/aspirate, nasal aspirates, mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider), and oropharyngeal swabs are acceptable, however, performance characteristics are unknown.
- The clinical performance has been established in specimens collected from subjects suspected of COVID-19 or subjects suspected of COVID-19 presenting with one or more symptoms of COVID-19. Performance of specimens collected from individuals without symptoms or other epidemiological reasons to suspect COVID-19 when tested at least weekly and with no more than 168 hours between tests 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.
- The clinical performance has not been established in 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.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The RNase P endogenous control is included to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- Good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.
- For BD MAX™ TNA extraction: Tobramycin at $1.1x10^{-3}$ g/Sample Buffer Tube interferes with the assay. Lower concentrations of Tobramycin have not been evaluated.
- The effect of homeopathic medications for respiratory symptoms on the assay performance was not tested.
- BD SARS-CoV-2 Reagents have not been evaluated for patients receiving intranasally administered influenza vaccine.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The BD SARS-CoV-2 Reagents for BD MAX™ System 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/vitro-diagnostics-euas

To assist clinical laboratories running the BD SARS-CoV-2 Reagents for BD MAX™ System, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories* using the BD SARS-CoV-2 Reagents for BD MAX™ System must include with result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the BD SARS-CoV-2 Reagents for BD MAX™ System must use the BD SARS-CoV-2 Reagents for BD MAX™ System as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized ancillary reagents, and authorized material required to perform the BD SARS-CoV-2 Reagents for BD MAX™ System test are not permitted.
- Authorized laboratories that receive the BD SARS-CoV-2 Reagents for BD MAX™ System test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- All laboratory personnel using the BD SARS-CoV-2 Reagents for BD MAX™ System test must be appropriately trained in RT-PCR techniques, use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Authorized laboratories using the BD SARS-CoV-2 Reagents for BD MAX™ System test must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Becton, Dickinson and Company, authorized distributors, and authorized laboratories using the BD SARS-CoV-2 Reagents for BD MAX™ System must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.
- Authorized laboratories will collect information on the performance of the BD SARS-CoV-2 Reagents for BD MAX™ System test and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Becton, Dickinson and Company Customer Technical Support 1.800.638.8663 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the BD SARS-CoV-2 Reagents for BD MAX™ System test of which they become aware.

*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 moderate or high complexity tests” as “authorized laboratories”.

NON-CLINICAL PERFORMANCE EVALUATION

Limit of Detection (LoD)

LoD studies determine the lowest detectable concentration of the SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive.

To determine the LoD, quantified heat-inactivated virus, obtained from ATCC® (Catalog No. VR-1986HK) was serially diluted into pooled negative nasopharyngeal clinical matrix, a total of 5 concentrations levels, with 2-fold serial dilutions between each level.

Confirmation of the estimated LoD was performed with one reagent lot in replicates of 20 prepared in pooled nasopharyngeal swab clinical matrix. The LoD is the lowest concentration (reported as genomic copies/mL, GC/mL) of SARS-CoV-2 that can be reproducibly distinguished from negative samples ≥95%. The LoD for the assay is 640 GC/mL.

Table 3. LoD determination of SARS-CoV-2 USA-WA1/2020 heat-inactivated virus

Strain	Concentration	Total Valid Results	Positives			Mean Ct		
			CoV-2	N1	N2	N1	N2	RNase P
USA-WA1/2020 Heat-Inactivated Virus (Stock 3.75e+05 GC/mL)	640 GC/mL	20	20	20	20	33.2	33.2	21.0

Additionally, 20 replicates at 40 genomic equivalents/ml (GE/mL) using quantified genomic viral RNA from SARS-CoV-2 obtained from BEI Resources (Catalog No. NR52285) were tested and 20 positive results were obtained.

Table 4. Detection of quantified genomic viral RNA from SARS-CoV-2 USA-WA1/2020

Strain	Concentration	Total Valid Results	Positives			Mean Ct		
			CoV-2	N1	N2	N1	N2	RNase P
USA-WA1/2020 Genomic RNA (Stock 4.8e+07 GE/mL)	40 GE/mL	20	20	20	20	32.2	32.6	21.5

Reactivity/Inclusivity

An *in silico* comparison of the N1 and N2 primer sets was performed using all available high quality SARS-CoV-2 sequences submitted to the GISAID EpiCoV database by January 13, 2021 (n=329,434). Alignments against the N gene showed that both N1 and N2 primer/probe sets are a perfect match to 93.8% of sequences in the database, 96.8% of the sequences were a perfect match to the N1 primer set region, and 97.0% were a perfect match to the N2 primer set region. In total, 99.9% are a perfect match to either the N1 or the N2 region primer set.

Cross-Reactivity

The nCoV N1 and nCoV N2 primers and probes utilized within the BD SARS-CoV-2 Reagents for BD MAX™ System are identical in sequence to those reported in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The CDC reported an *in silico* analysis of primer and probe sequences within their IFU (CDC-006-0019, Rev 02), and has been copied below for reference:

BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 10/03/2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2, respectively.

2019-nCoV_N1 Assay:

Probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

2019-nCoV_N2 Assay:

The forward primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is no prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

CLINICAL EVALUATION

The clinical performance of BD SARS-CoV-2 Reagents for BD MAX™ System with retrospective collected nasopharyngeal swab samples was evaluated using 100 individual negative clinical samples and 30 positive clinical samples as determined by an FDA-authorized SARS-CoV-2 molecular assay. Clinical samples were collected from patients suspected of COVID-19 by their healthcare provider and tested in blinded and randomized fashion.

The positive samples showed 100% agreement with the expected results. The negative samples showed 97% agreement with the expected results.

Table 5. Clinical Comparison with an FDA-authorized molecular assay

		FDA-authorized molecular assay	
		Positive	**Negative**
BD SARS-CoV-2 Reagents	**Positive**	30	3
	Negative	0	97
	Total	30	100
Positive Percent Agreement:		100% (95% CI: 88.7%–100%)	
Negative Percent Agreement:		97% (95% CI: 91.5%–99.0%)	

The performance of BD SARS-CoV-2 Reagents for BD MAX™ System was further evaluated using 20 contrived positive clinical samples prepared in negative nasopharyngeal matrix. Low positive contrived clinical samples were prepared by spiking quantified heat-inactivated virus (USA-WA1/2020 strain) into individual negative clinical matrix to approximately 2x LoD. The low positive samples showed 100% agreement with the expected results.

Table 6. Clinical Evaluation with Contrived Nasopharyngeal Swab Samples

Concentration	**Total Valid Results**	**Positive**			**Mean Ct**		
		CoV-2	**N1**	**N2**	**N1**	**N2**	**RNase P**
2x LoD	20	20	20	20	32.1	32.0	23.2

The performance of BD SARS-CoV-2 Reagents for BD MAX™ System with nasal swab matrix expressed in UVT or 0.85% saline was evaluated using 15 contrived positive samples and 5 negative samples prepared in negative nasal clinical matrix.

Low positive and moderate positive contrived clinical samples were prepared by spiking quantified heat-inactivated virus (USA-WA1/2020 strain) into individual negative clinical matrix at concentrations of 2x LoD (10 samples) and 5x LoD (5 samples), respectively. The low positive, moderate positive, and negative samples showed 100% agreement with the expected results.

Table 7. Clinical Evaluation with Contrived Nasal Swab Samples

Sample Type	**Sample Concentration**	**Positive**			**Mean Ct**		
		SARS-CoV-2	**N1**	**N2**	**N1**	**N2**	**RNase P**
UVT	Negative	0% (0/5)	0% (0/5)	0% (0/5)	N/A	N/A	24.6
	Low Positive (2x)	100% (10/10)	100% (10/10)	100% (10/10)	31.8	32.0	24.9
	Moderate Positive (5x)	100% (5/5)*	100% (5/5)	100% (5/5)	30.5	30.6	23.9
Saline	Negative	0% (0/5)	0% (0/5)	0% (0/5)	N/A	N/A	25.5
	Low Positive (2x)	100% (10/10)	100% (10/10)	100% (10/10)	32.4	32.9	26.3
	Moderate Positive (5x)	100% (5/5)	100% (5/5)	100% (5/5)	30.9	31.6	26.0

*One invalid result was obtained during initial testing. The expected result was obtained upon re-test of the sample.

Post Market Clinical Evaluation Study

A prospective clinical study was conducted from September 2020 – January 2021 to evaluate the clinical performance of the BD SARS-CoV-2 Reagents for BD MAX™ System compared to an FDA authorized SARS-CoV-2 molecular assay. Nasopharyngeal specimens were collected from subjects suspected of COVID-19 or subjects suspected of COVID-19 presenting with one or more symptoms of COVID-19 at four geographically diverse clinical sites in the United States. The specimens were stored in viral transport media and tested at one of two clinical centers using the BD SARS-CoV-2 Reagents for BD MAX™ System and the FDA authorized SARS-CoV-2 molecular assay. Samples with initial BD MAX™ non-reportable results (unresolved, indeterminate, or incomplete) were repeated with a new aliquot of viral transport media.

One site enrolled specimens from subjects 1 month and older; three sites enrolled subjects 18 years and older. The population consisted of 40% males and 60% females. Of the 709 specimens tested on the BD MAX™ System, one specimen had a final result of unresolved and is not included in the data analysis. There were 708 compliant specimens with a paired BD MAX™ SARS-CoV-2 result and an FDA authorized molecular assay result for analysis. Discrepant specimens were tested with up to two additional FDA authorized SARS-CoV-2 nucleic acid amplification assays.

The positive, negative, and overall percent agreement and the 95% confidence intervals of the BD SARS-CoV-2 Reagents for BD MAX™ System compared to an FDA EUA SARS-CoV-2 nucleic acid amplification assay is shown in Table 8.

Table 8: Percent Agreement of BD SARS-CoV-2 Reagents for BD MAX™ System vs. FDA Authorized Molecular Assay
FDA Authorized Molecular Assay

	FDA Authorized Molecular Assay		
BD SARS-CoV-2 Reagents for BD MAX™ System Result	**Positive**	**Negative**	**Total**
Positive	138	19[a]	157
Negative	0	551	551
Total	138	570	708
Positive Percent Agreement:	100% (95% CI: 97.3%–100.0%)		
Negative Percent Agreement:	96.7% (95% CI: 94.9%–97.9%)		

[a] Five (5) of the 19 BD SARS-CoV-2 Reagents for BD MAX™ System positive/EUA comparator negative specimens were positive by at least one additional FDA authorized SARS-CoV-2 nucleic acid amplification assay. Two additional specimens were considered SARS-CoV-2 presumptive positive by one method.

Non-Reportable Results

In the clinical evaluation study, the initial total non-reportable rate representing unresolved, indeterminate, and incomplete results was 1.4% (10/709; 95% CI: 0.8–2.6%). Following a valid repeat test, 0.1% (1/709; 95% CI: 0.0–0.8%) specimens remained non-reportable.

Table 9: Non-reportable Rates[a]

Unresolved Rate		Indeterminate Rate		Incomplete Rate		Total Non-reportable Rate	
Initial (95% CI)	**Final (95% CI)**	**Initial (95% CI)**	**Final (95% CI)**	**Initial (95% CI)**	**Final (95% CI)**	**Initial (95% CI)**	**Final (95% CI)**
1.4% (10/709) (0.8%, 2.6%)	0.1% (1/709) (0.0%, 0.8%)	0.0% (0/709) (0.0%, 0.5%)	0.0% (0/709) (0.0%, 0.5%)	0.0% (0/709) (0.0%, 0.5%)	0.0% (0/709) (0.0%, 0.5%)	1.4% (10/709) (0.8%, 2.6%)	0.1% (1/709) (0.0%, 0.8%)

[a] Note that samples associated with BD MAX™ System runs with an external control failure are designated as invalid (INV). Of the 709 total samples tested, there were 64 initial INV results due to external control failures. The samples were successfully repeated with either a positive or negative result.

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 BD MAX™ System. The results are summarized in Table 10.

Table 10. 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 matrix	5,400 NDU/mL	N/A
MERS-CoV		N/A	ND

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

REFERENCES

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to the latest edition).
2. Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Chosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC) 21–1112.
3. BD MAX System User's Manual (refer to the latest revision) BD Life Sciences, Sparks, MD 21152 USA.
4. Centers for Disease Control and Prevention. Interim guidelines for collecting, handling, and testing clinical specimens from persons for Coronavirus Disease 2019 (COVID-19).

Change History

Revision	Date	Change Summary
(05)	2020-09	Clarified CLIA statements and that all (not just positive) results must be reported to the appropriate public health authorities. Updated FDA website.
(06)	2021-03	Updated Warnings and Precautions section. Added frozen storage information in Instructions for Use. Updated Limitation of the Procedure section to remove presumptive positive limitation, add limitation regarding performance with variants, and remove duplicated authorization verbiage. Updated FDA website address. Added *in silico* comparison to Reactivity/ Inclusivity section. Added Post Market Clinical Evaluation Study section. Made formatting and typographical updates.
(07)	2021-04	Updated Intended Use and Limitations of the Procedure to include serial testing of asymptomatic individuals. Made formatting and typographical updates.

SYMBOLS GLOSSARY [L006715(05) 2021-04]

Some symbols listed below may not apply to this product.

US Customers only: For symbol glossary, refer to bd.com/symbols-glossary

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Date of manufacture
	Use-by date
	Batch code
	Catalogue number
	Serial number
	Sterile
	Sterilized using aseptic processing techniques
	Sterilized using ethylene oxide
	Sterilized using irradiation
	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged
	Sterile fluid path
	Sterile fluid path (ethylene oxide)
	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult instructions for use For electronic instructions for use, the url accompanies the symbol.
	Caution
	Contains or presence of natural rubber latex
	*In vitro* diagnostic medical device
	Negative control
	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
	Patient number

Symbol	Meaning
	This way up
	Do not stack
	Single sterile barrier system
	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
	Meets FCC requirements per 21 CFR Part 15
	UL product certification for US and Canada
	Unique device identifier

BD

Technical Service and Support: In the United States contact BD at 1.800.638.8663 or bd.com.

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Preparation of External Positive and Negative Controls for BD SARS-CoV-2 Reagents for BD MAX™ System

Intended Use

The BD SARS-CoV-2 Reagents for BD MAX™ System is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, anterior nasal, mid-turbinate, and oropharyngeal swab specimens, nasopharyngeal wash/aspirate or nasal aspirates obtained from individuals who are either suspected of COVID-19 by their healthcare provider, or from individuals without symptoms or other epidemiological 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 moderate or high complexity tests.[1]

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory samples 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.[1]

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.[1]

The BD SARS-CoV-2 Reagents for BD MAX System is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR, *in vitro* diagnostic procedures, and use of the BD MAX System. The BD SARS-CoV-2 Reagents for BD MAX System is only for use under the Food and Drug Administration's Emergency Use Authorization.[1]

Purpose

According to the "Guidance for COVID-19 Testing for CAP-Accredited Laboratories" by the College of American Pathologists, clinical laboratories using EUA assays for COVID-19 testing must perform quality control each of day of patient testing.[2] External positive and negative controls are samples that act as surrogates for clinical specimens. They are processed like a clinical specimen to monitor the ongoing performance of the entire analytic process in every assay run.

The purpose of this document is to provide clinical laboratories with suggested procedures for preparing External Positive Controls (EPC) and External Negative Controls (ENC) for the BD SARS-CoV-2 Reagents on the BD MAX System using commercially prepared controls and materials.

Table 1. Commercially Prepared Controls for SARS-CoV-2

Manufacturer	SARS-CoV-2 Control	Other Control
BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates[3]	SARS-CoV-2 Nucleocapsid Phosphoprotein gene N1 SARS-CoV-2 Nucleocapsid Phosphoprotein gene N2	RNase P
Microbiologics® Helix Elite™ Synthetic RNA Standard[4]	1044 nucleotide segment of SARS-CoV-2 N (nucleocapsid) gene (N1, N2 and N3)	Negative Cellularity Control
Integrated DNA Technologies Positive Control Plasmids[5,6]	Complete 2019-nCoV Nucleocapsid gene (N1, N2 and N3)	Portion of the RPP30 single copy gene found in humans

Each suggested procedure for preparing an EPC or ENC has been verified by BD. However, the choice of EPC and ENC for the BD SARS-CoV-2 Reagents for BD MAX System is ultimately the decision of the laboratory, in accordance with applicable local, state, and/or federal regulations, accreditation requirements and the laboratory's standard Quality Control (QC) procedures.

A) BioGX SARS-CoV-2 Nucleocapsid N1 and N2 genes and RNase P

The BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates kit contains lyophilized control template beads for the SARS-CoV-2 Nucleocapsid Phosphoprotein gene (N1), SARS-CoV-2 Nucleocapsid Phosphoprotein gene (N2), and RNase P.[3] The beads are formulated with quantified RNA (single stranded RNA) at 1 x 10^5 copies per tube in a lyophilized bead format. Each product package contains 1 pouch of 24 tubes with each tube containing 1 lyophilized control template bead.[3] The BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates should be stored at 2-8°C.[3]

Table 2. Materials Needed for the BioGX External Positive Controls

Material	Part #
BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates	BD # 444214
BD MAX ExK TNA-3 Sample Buffer Tubes	BD # 442827 or 445003-01
Nuclease-free water*	Invitrogen # 4387936**

**Alternate diluents e.g., Universal Transport Media (UTM) may be used for preparation of the Sample Buffer Tube if validated by the laboratory*

***or Equivalent*

Preparation of External Positive Controls (EPC) and External Negative Controls (ENC) from BioGX Control Beads

1. To prepare the SARS-CoV-2 N1 and N2 EPC, pipet 650 µL of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
2. Rehydrate the BioGX SARS-CoV-2 N1 and N2 positive control beads individually with 100 µL of nuclease-free water.
3. Pipette the entire volume of the rehydrated N1 and N2 positive controls into the **same** SBT. Close

the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the SARS-CoV-2 N1/N2 EPC.

4. To prepare the RNase P positive control bead, repeat steps 1-3 above with a **new** BD MAX ExK TNA-3 SBT and the BioGX RNase P positive control beads. The rehydrated RNase P positive control bead is added to the separate SBT.
5. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the tube as the RNase P ENC.
6. Proceed with testing the prepared SARS-CoV-2 N1/N2 EPC SBT and the RNase P ENC SBT with the BD SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.[1]

Expected Results

Laboratories should refer to the BD SARS-CoV-2 Reagents for BD MAX System Package Insert for full interpretation of external control test results.[1] The expected results are below.

Table 3. Expected results for the SARS-CoV-2 N1 / N1 and RNase P External Controls

External Positive Control	N1	N2	RNase P	COV-2	Result Interpretation
SARS CoV-2 N1/N2 EPC	Pos	Pos	N/A	Pos	Positive
RNase P ENC	Neg	Neg	Pos	Neg	Positive

The SARS CoV-2 N1/N2 EPC and RNase P ENC should yield the expected positive results.

B) Microbiologics SARS-CoV-2 Synthetic RNA (N gene targets) and Negative Cellularity Control (RNaseP target)

The Microbiologics® Helix Elite™ SARS-CoV-2 synthetic RNA standard is a 1044-nucleotide portion of the SARS-CoV-2 N (nucleocapsid) gene containing the three markers N1, N2 and N3. Each kit includes 1 vial of dried synthetic RNA, 1 vial of molecular standard water, and a certificate of analysis.[4] The Helix Elite™ SARS-CoV-2 synthetic RNA standard should be stored at 2-25°C according to the manufacturer's instructions.[4] The Microbiologics® Helix Elite™ Negative Cellularity Control is specifically designed, full process controls containing A549 lung epithelial cells. Each kit contains 5 lyophilized pellets, packaged in individual vials[5]. The Negative Cellularity control serves as an RNaseP external negative control (ENC).

Table 4. Materials Needed for the Microbiologics External Positive Control

Material	Part #
Microbiologics® Helix Elite™ Synthetic Standard SARS-CoV-2 Synthetic RNA (N gene Targets)	Microbiologics # HE0060S
Microbiologics® Helix Elite™ Negative Cellularity Control (RNase P target)	Microbiologics #HE0058N
BD MAX ExK TNA-3 Sample Buffer Tubes	BD # 442827 or 445003-01
Molecular standard or Nuclease-free water*	Invitrogen # 4387936**
1X TE buffer, pH 8.0	Thermo Fisher Scientific # AM9849**
2 mL Tubes	VWR # 10025-756**

**Alternate diluents e.g., Universal Transport Media (UTM) may be used for preparation of the Sample Buffer Tube if validated by the laboratory*

***or Equivalent*

Preparation of SARS-CoV-2 Synthetic RNA External Positive Control from Microbiologics Standard

1. To prepare the SARS-CoV-2 Synthetic RNA EPC, add 750 µL of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
2. Rehydrate the lyophilized powder of the Helix Elite™ SARS-CoV-2 Synthetic RNA standard and dilute per the manufactures instructions[4].
   a. Add 55 µL of nuclease-free water for a concentration stock.
   b. Dilute the rehydrated SARS-CoV-2 Synthetic RNA by transferring the 90 µL of nuclease-free water to 10 µL of the concentrated stock.
3. Prepare the spiking dilution by adding 94.5 µL of nuclease-free water to 5.5 µL of the diluted stock.
4. Pipette 50 µL of the diluted stock into the SBT.
5. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the tube as the SARS-CoV-2 Synthetic RNA EPC.
6. Proceed with testing the prepared SARS-CoV-2 Synthetic RNA EPC with the BD SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.[1]

Preparation of Negative Cellularity Control (RNaseP ENC) from Microbiologics

1. To prepare the Negative Cellularity Control, add 750 µL of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
2. Rehydrate the lyophilized powder of the Helix Elite™ Negative Cellularity Control and dilute per the manufactures instructions[5].
3. Add the rehydrated control to the sample buffer tube prepared in the step above.
4. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the tube as the RNaseP EPC.
5. Proceed with testing the prepared RNaseP ENC with the BD SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.[1]

Expected Results

Laboratories should refer to the BD SARS-CoV-2 Reagents for BD MAX System Package Insert for full interpretation of external control test results.[1] The expected results are below.

Table 5. Expected results for the SARS-CoV-2 Synthetic RNA External Positive and Negative Controls

External Positive Control	N1	N2	RNase P	COV-2	Result Interpretation
SARS-CoV-2 Synthetic RNA EPC	Pos	Pos	N/A	Pos	Positive
Negative Cellularity Control (RNaseP ENC)	Neg	Neg	Pos	Neg	Positive

The SARS-CoV-2 Synthetic RNA EPC and Negative Cellularity Control (RNaseP ENC) should yield the expected positive results.

C) IDT 2019-nCoV_N and Hs_RPP30 Positive Control Plasmids

The Integrated DNA Technologies (IDT) 2019-nCoV_N Positive Control plasmid contains the complete nucleocapsid gene from 2019-nCoV (SARS-CoV-2). The Hs_RPP30 Positive Control plasmid contains a portion of the RPP30 gene, a single copy gene present in the human genome.[6,7] The IDT control plasmids are derived from the CDC nCoV EUA kit. Control Plasmids are delivered at 250 µL (200,000 copies/µL) in IDTE, pH 8.0. Store at -20°C or colder.

Table 6. Materials Needed for the IDT External Controls

Material	Part #
IDT 2019-nCoV_N_Positive Control Plasmid	IDT # 10006625
IDT Hs_RPP30 Positive Control Plasmid	IDT # 10006626
ExK TNA-3 Sample Buffer Tubes	BD # 442827 or 445003-01
Nuclease-free water*	Invitrogen # 4387936**
1X TE, buffer pH 8.0	Thermo Fisher Scientific # AM9849**
2 mL Tubes	VWR # 10025-756**

**Alternate diluents e.g., Universal Transport Media (UTM) may be used for preparation of the Sample Buffer Tube if validated by the laboratory*

***or Equivalent*

Preparation of External Positive Controls (EPC) and External Negative Controls (ENC) from IDT Control Plasmids

1. To prepare the 2019-nCOV_N EPC add 750 µL of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
2. Prepare a dilution of the stock IDT 2019-nCoV_N Positive Control plasmid (200,000 copies/µL) in TE buffer (See Table 7 for dilution scheme).
3. Pipette 50 µL of the 200 copies/µL diluted stock into the SBT.
4. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the 2019-nCOV_N EPC.
5. To prepare the Hs_RPP30 EPC, repeat steps 1-3 above with a **new** BD MAX ExK TNA-3 SBT and the IDT Hs_RPP30 Positive Control Plasmid.
6. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the Hs_RPP30 ENC.
7. Proceed with testing the 2019-nCoV_N EPC SBT and the Hs_RPP30 ENC SBT with the BD SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.[1]

Table 7. IDT 2019-nCoV_N and Hs_RPP30 Plasmid Dilution Scheme

Stock (cps/µL)	Dilution Factor	Total Volume of Buffer (µL)	Volume of Stock to Spike (µL)	Volume of TE buffer (µL)	Dilution Conc (cps/µL)
200,000	10	50	5	45	20,000
20,000	10	100	10	90	2,000
2,000	10	500	50	450	200

Expected Results

Laboratories should refer to the BD SARS-CoV-2 Reagents for BD MAX System Package Insert for full interpretation of external control test results.[1] The expected results are below.

Table 8. Expected results for the 2019-nCoV_N and Hs_RPP30 External Controls

External Positive Control	N1	N2	RNase P	COV-2	Result Interpretation
2019-nCoV_N EPC	Pos	Pos	N/A	Pos	Positive
Hs_RPP30 ENC	Neg	Neg	Pos	Neg	Positive

The 2019-nCoV_N EPC and Hs_RPP30 ENC should yield the expected positive results.

Expanding the Protocols

The example protocols described above may be expanded at the laboratory's discretion by increasing the number of each EPC for each run of the BD SARS-CoV-2 Reagents for BD MAX System. Please follow the Storage and Stability instructions in the respective Package Inserts of all media and materials used.

IMPORTANT NOTE: Laboratories should follow Good Laboratory Practices and Universal Precautions at all times during preparation and use of external control materials. All materials should be disposed of properly as required by the institution.

Warnings

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

Technical Service and Support

BD is committed to providing our customers timely and accurate support. If there are any questions or concerns about this document or the contents, please contact BD Life Sciences – Integrated Diagnostic Solutions Technical Service and Support by dialing 1-800-638-8663 (U.S.).

References

(1) BD SARS-CoV-2 Reagents for BD MAX™ System Package Insert. Becton Dickinson and Company, Sparks, MD. (Latest version).
(2) College of American Pathologists "Guidance for COVID-19 Testing for CAP-Accredited Laboratories" https://www.cap.org/laboratory-improvement/news-and-updates/guidance-for-covid-19-testing-for-cap-accredited-laboratories Accessed March 21, 2020.
(3) BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates. Package Insert (Latest version)
(4) Microbiologics® Helix Elite™ Synthetic Standard SARS-CoV-2 Synthetic RNA (N Gene Targets). Package Insert (Latest version)
(5) Microbiologics® Helix Elite™ Negative Cellularity Control. Package Insert (Latest version)
(6) Integrated DNA Technologies 2019-nCoV_N_Positive Control Plasmid. Product Description (Latest version)
(7) Integrated DNA Technologies Hs_RPP30 Positive Control Plasmid. Product Description (Latest version)

BD SARS-CoV-2 Reagents for BD MAX™ System
SARS-CoV-2/Flu for BD MAX™ System

REF 445003-01 REF 445011
P0259(02)

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