CE

Truemix™

COVID-19

Real Time Duplex PCR Test for COVID -19

1. INTENDED USE

Truemix™ COVID-19 (**REF** 701430008/701430024/701430048/701430096) is a lyophilised, ready-to-use, open format, duplex Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the qualitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swabs and aids in the diagnosis of COVID-19. The test detects the *E* and *Orf1a* genes of the virus. **Truemix™ COVID-19** runs on standard Real Time PCR analyzers.

2. INTRODUCTION

SARS CoV-2 is the causative agent for corona virus disease 2019 or COVID-19 in Humans. SARS CoV-2 is a Beta Corona Virus, one of the four genera of Corona Viruses. Coronaviruses are enveloped non-segmented positive sense RNAviruses belonging to the family coronaviridae and the order Nidovirales and broadly distributed in humans and other mammals. The common signs of COVID-19 infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Early and correct identification of infection with SARS CoV-2 is important for effective isolation, treatment and case management of COVID-19. In line with WHO recommendations, molecular diagnostics are currently the method of choice for such virus detection and differentiation.

3. PRINCIPLE OF THE TEST

Truemix™ COVID-19 is a freeze dried, ready-to-use open format assay, works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. The assay targets *E* and *Orf1a* genes of SARS CoV-2 virus and also includes an internal control in each tube (human *RNase P* gene target), to verify the validity of sample collection, extraction and PCR. Reverse Transcription Real-Time RT PCR technology utilizes reverse-transcriptase (RT) to convert RNA of SARS CoV-2 virus into complementary DNA (cDNA), and then polymerase chain reaction (PCR) for the amplification of specific *E* and *Orf1a* gene target sequences. Real time detection of above process is enabled using target specific fluorescent hydrolytic probes.

The test consists of three processes in a single tube assay:

· Reverse transcription of RNA to cDNA, followed by

· PCR amplification of targets and Internal Control

· Simultaneous, real time detection by dual labeled fluorescent probes

4. TARGET SELECTION

The target sequence for this kit has been taken from the *E* and *Orf1a* gene of SARS CoV-2 and human *RNase P*. Detection of the human *RNase P* gene serves as a full process internal positive control (IPC) for proper swab collection, nucleic acid extraction and PCR.

5. CONTENTS OF THE Truemix™ COVID-19 KIT

A. **Truemix™** Pouch containing

1. Strip of 8 dried down PCR mix
2. Desiccants

B. Accessory Pack containing

1. Reconstitution Buffer
2. Positive Control (Dried Down)
3. Negative Control

C. Strip of 8 PCR Flat Caps

D. Package Insert

REF	701430008	701430024	701430048	701430096
Σ	8T	24T	48T	96T

6. STORAGE AND STABILITY

Truemix™ COVID-19 is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for upto one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

7. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

a. Cold rack for PCR setup
b. Pipettes and Filter barrier tips
c. PCR strip centrifuge
d. Waste disposal bins

8. SAFETY PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Do not use kit beyond expiry date.
3. Carefully read the User Manuals, package inserts and Material safety Data Sheets (MSDS) of all the components of the **Truemix™ COVID-19** before use.
4. All materials of human origin should be handled as though potentially infectious.
5. Do not pipette any material by mouth.
6. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
7. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

9. PROCEDURAL PRECAUTIONS

1. Do not exchange kit components from different lots.
2. Check all packaging before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However if the outer packaging is damaged the user must check that components of the kit are intact before using them.
3. Do not perform the assay in the presence of reactive vapours (e.g. from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
4. All pipetting steps should be performed with utmost care and accuracy. Cross-contamination between reagents and samples may invalidate results.

10. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truemix™** assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. It is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or specimens.
4. A specimen for which the **Truemix™** assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truemix™** assay should be interpreted in the context of other clinical and laboratory findings.

11. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared Sodium hypochlorite [10 times dilution of 5% Sodium hypochlorite (household bleach)] before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves should be disposed off as potentially bio-hazardous waste e.g. in a biohazard waste container.

12. COMPATIBLE REAL-TIME PCR INSTRUMENTS

Truemix™ COVID-19 kit will be compatible for real time PCR analyzer with 0.2mL PCR tubes. This kit can be used in other real time PCR by transferring reconstituted contents to the tubes which is compatible with the respective PCR machines. **Truemix™ COVID-19** kit has been validated for use with QuantStudio™ 5 Real Time PCR (Applied Biosystems™), ABI 7500 (Applied Biosystems™), Rotor-Gene Q5plex (Qiagen), Light Cycler 96 Instrument (Roche), CFX96 Real-Time PCR Detection System (Bio-Rad).

13. TEST PROCEDURE

A. Sample Preparation:

Viral RNA has to be extracted from the swab medium (Viral Transport Medium) as per guidelines. Qiagen viral mini kit or other equivalents recommended by agencies can be used. Lab should verify the suitability of the prep kit used for this application.

Note: Internal control used in this kit is Human *RNase P* gene target, which comes from the swab. No additional control to be added to sample, before prep.

B. Positive Control Preparation:

The dried down positive control in the micro tube needs to be reconstituted. Pipette 50 µl of negative control from the negative control vial into the micro tube of positive control using a fresh DNase/ RNase free filter barrier micropipette tip. Dispose off the tip immediately as per the section on "Disposal and Destruction" (Section 17). Mix the micro tube gently for a few minutes. The reconstituted positive control is ready to use. It can be stored at -20°C for further use. The negative control is a ready to use solution.

⚠ Caution: Positive control can cause contamination and should be handled with extreme care while opening the micro tube, reconstitution and use. Avoid spillage. Dispose and destroy the micro tube with left over control and micro pipette tips as described in section 17.

C. Master Mix Setup: :

The kit provides PCR mastermix as a ready-to-use, freeze dried format. Before adding sample (RNA), add 5 µl of provided reconstitution buffer to each tube.

Per Tube	Volume (µL)
Reconstitution buffer	5
Elute/Positive Control/Negative Control/Internal control	5
Total	10

D. Reaction Setup:

1. Place the tube strip with dry reagents on a cold rack. [Pre-cool the rack by storing it in freezer compartment].
2. Further steps need to be done as quickly as possible. Keep temperature between 0-10°C, till the tubes are placed in PCR instrument.
3. Remove the rubber bungs from the tubes and discard.
4. Pipette 5 µL of the reconstitution buffer, provided in the kit, to each PCR tube.
5. Pipette 5 µL of the elute to each PCR tube.
6. Close the tube with PCR flat caps provided along with the kit & spin down for approximately 30 seconds, to bring down the liquids.

Note: Use calibrated pipettes, with filter barrier tips. Change tip for each sample and discard used tips directly into freshly prepared 0.5% Sodium hypochlorite solution.

14. SETTING UP THE REAL-TIME PCR INSTRUMENT

For basic information regarding the setup and programming of the real-time PCR instruments, please refer to the user manual of the respective instrument.

A. Plate settings:

Settings	
Reaction Volume	10µL
Ramp Rate	Default

B. Fluorescence Detectors (Dyes):

Description	Gene Target	Reporter channel
Screening target	*E* gene	ROX / equivalent
Confirmatory target	*Orf1a* gene	FAM /equivalent
Internal Control	*RNaseP* gene	Cy5 / equivalent

C. Temperature profile and data acquisition:

	Stage	Cycle Repeats	Data Acquisition	Temperature[°C]	Time [seconds]
Reverse Transcription	Hold	1	-	50	300
Initial Denaturation	Hold	1	-	95	60
Amplification	Cycling	40	-	95	10
			Yes	57	34

Note: *Quencher & passive control can be chosen as "none"*

15. DATA ANALYSIS

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

16. RESULTS & INTERPRETATIONS

Qualitative Screening:

Detection Channel			Result Interpretation	Action
Orf1a	*E*	*RNase P*		
+	+	+/-	SARS CoV-2 POSITIVE	Report Positive
+	-	+/-	SARS CoV-2 POSITIVE	Report Positive
-	+	+/-	SARS CoV-2 PRESUMPTIVE POSITIVE	Repeat after 48-72 hours
-	-	+	SARS CoV-2 NEGATIVE	Report Negative
-	-	-	INVALID	Collect new swab and repeat

Ct values above 38 should be considered as Not Detected

17. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as microtube, microtube cap, rubber bungs, pipette tips, positive control tube etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

18. QUALITY CONTROL

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. A negative control, positive control and internal control should be set for each lot.

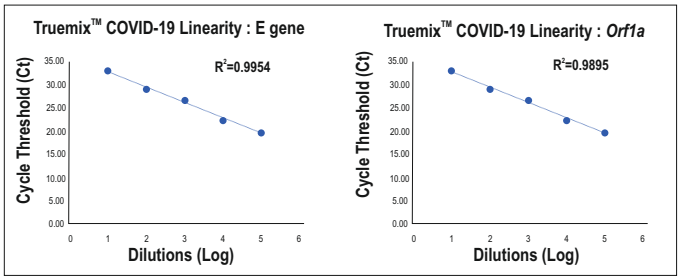
19. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Exclusivity (Primer specificity): The following viruses and microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine potential cross-reactivity in the **Truemix™ COVID-19** assay. Results obtained showed no potential cross reactivity of the **Truemix™ COVID-19** assay with the listed organisms.

Bacteria	Viruses
Chlamydia pneumoniae	Human Coronavirus 229E
Haemophilus influenzae	Human Coronavirus OC43
Legionella pneumophila	Human Coronavirus HKU1
Mycobacterium tuberculosis	Human Coronavirus NL63
Streptococcus pneumoniae	MERS - Coronavirus
Streptococcus pyogenes	Human adenovirus B1
Bordetella pertussis	Human Metapneumovirus (HMPV)
Mycoplasma pneumoniae	Parainfluenza virus 1 - 4
Corynebacterium diphtheriae	Human respirovirus 1
Bacillus anthracis (Anthrax)	Influenza A
Moraxella catarrhalis	Influenza B
Neisseria elongata	Enterovirus D68
Neisseria meningitidis	Respiratory syncytial virus
Pseudomonas aeruginosa	Rhinovirus
Staphylococcus epidermidis	Influenza C
Streptococcus salivarius	Parechovirus
Leptospira interrogans	**Fungi**
Chlamydia psittaci	*Pneumocystis jirovecii* (PJP)
Coxiella burnetii	*Candida albicans*

Linearity & PCR Efficiency:

Using the dilution series from **Trueprep® AUTO** elutes run on **Truemix™ COVID-19**, log linear curve was plotted to check the linearity of Ct values on **Truemix™ COVID-19** test.

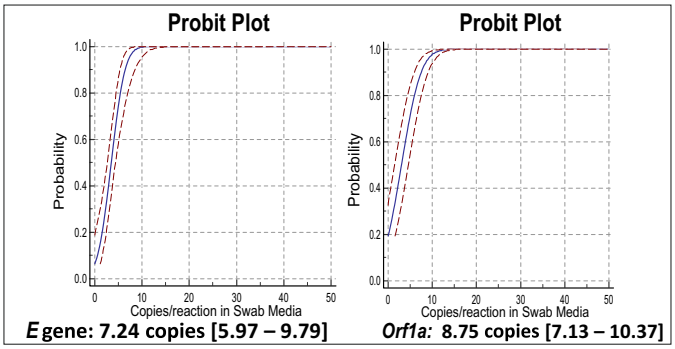


Linearity and PCR efficiency on **Truemix™ COVID-19**. Y axis indicate Ct values and X axis is arbitrary log numbers indicating dilutions.

Conclusion: Slope of the curve is *Orf1a* (-3.353) and *E* gene (-3.348). Assay was observed to be linear over the range of dilutions tested and PCR efficiency was found to be 98.72 % for *Orf1a* and 98.92% for *E* gene. **Truemix™ COVID-19** assay detected *E* gene and *Orf1a* targets up to dilution 10^{-5}(D5) with valid Ct value.

Limit of Detection (LoD):

The limit of detection (LoD) was estimated using AccuPlex™ SARS-CoV-2 Verification Panel (Seracare, 0505-0168), as per CLSI EP-17A guidelines. Briefly, six dilutions of quantified material were done in **Trueprep® AUTO** Transport Medium for Swab Specimen Pack. Each dilution was extracted using Qiagen mini and tested on the ABI 7500 real time PCR instrument. Probit regression was used to estimate LOD at 95% positivity, as indicated below.



***E* gene: 7.24 copies [5.97 – 9.79]** ***Orf1a:* 8.75 copies [7.13 – 10.37]**

LoD of *E* gene and *Orf1a* was estimated to be 7.24 copies/reaction & 8.75 copies/reaction with 95% CI interval of [5.97 – 9.79] and [7.13 – 10.37].

Cross reactivity:

Cross reactivity of **Truemix™ COVID-19** assay was evaluated by testing quantified samples of Hepatitis C Virus (Load:3.51x10^6 IU/mL), Hepatitis B Virus (Load:955000 IU/mL) and Human Immunodeficiency Virus (Load:8.4x10^4 IU/mL) No cross reactivity was observed with HCV, HIV and HBV.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truemix™ COVID-19** assay using three different titres of samples on ABI Real Time PCR systems. High, Medium and low titre samples were extracted on Qiagen RNA Mini and tested among three different users (Inter user), on two different devices (Inter device) and for 5 consecutive days (Inter day) to check the variability. Mean %CV values for all titres has been calculated for *Orf1A* gene as Inter User (2.09), Inter day (1.65) and Inter Device (1.59) and for *E* gene as Inter User (1.37), Inter day (1.10) and Inter Device (1.17) which were in the accepted range of ≤15% CV for **Truemix™ COVID-19** assay.

Precision:

Precision was tested by performing **Truemix™ COVID-19** assay of extracted RNA of High, Medium and Low titre for five consecutive days. Every day PCR for each titre RNA was run in triplicates. The %CV values obtained for High titre (2.29), Medium titre (1.69) and low titre (1.59) for *Orf1A* gene while High titre (2.34), Medium titre (1.52) and low titre (1.30) for *E* gene were within the accepted range of ≤15% CV for **Truemix™ COVID-19** assay.

Clinical Evaluation:

Clinical evaluation of **Truemix™ COVID-19** was done at ICMR-National Institute of Virology, Pune, India. Totally, 75 SARS CoV-2 positive and 85 SARS CoV-2 negative samples were tested.

		SARS CoV 2 Real-time PCR Test		
		Positive	Negative	Total
Truemix™ COVID-19	Positive	75	1	76
	Negative	0	84	84
	Total	75	85	160

Sensitivity and specificity for **Truemix™ COVID-19** was calculated against reference assay and found to be 100% (95% CI: 95.1-100) and 98.8% (95% CI: 93.6-99.8) respectively.

20. REFERENCES

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4. WHO. Middle East respiratory syndrome coronavirus (MERS-CoV). November, 2019. http://www.who.int/emergencies/mers-cov/en/ (accessed Jan 19, 2020).
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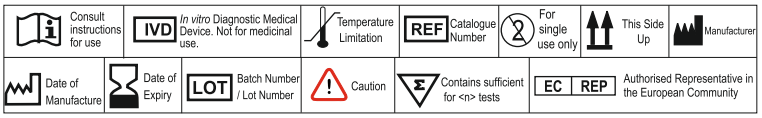
SYMBOL KEYS



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