

SN CoV Ensure

One Step Multiplex SARS CoV-2 RT-PCR

Detection Kit



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A NEW GENERATION COMPANY

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SN CoV Ensure

One Step Multiplex SARS CoV-2 RT-PCR Detection Kit

Description of the Product:

The **SN CoV Ensure One Step Multiplex SARS CoV-2 RT-PCR Detection Kit** constitutes a ready to use system for multiple detection (presence or absence) using polymerase chain reaction (PCR) in the Real Time PCR machine. The Specific Master mix contains reagents and enzymes for the specific amplification of SARS CoV-2 genes (**RdRp Gene & E Gene**) to generate amplified product and for the direct detection of the specific amplicon in fluorescence channel **FAM (E gene) & Cy5 (RdRp Gene)** of the Real Time PCR & the reference gene / Inhibition Control Gene **RNase P** on **HEX**.

Intended to use:

RT-PCR based diagnostic kit for the quantitative detection of SARS CoV-2 from nasopharyngeal, oropharyngeal swab samples from patients. It is recommended to use standard viral RNA extraction kit (spin column based or magnetic bead based) for isolation of viral RNA. The kit will also work on the automated RNA purification system. The kit intended to use by trained laboratory technician.

1. Contents of Kit:

Components	Color Code	Volume
SN CoV Ensure RT Master Mix (MM)	Natural	1000µL
PP Mix	Amber Tube	200µL
Positive control (PC)	Purple	100µL
Nuclease free water (NC)	White	100µL

***The Positive Control contains both targets; SARS CoV-2 RdRp & E Gene.**

All Vials have Color Coder tops to distinguish between different reagents.

Storage:

The kit is shipped and stored at -20°C

❖ Sample which can be used for extraction:

Nasopharyngeal Swab, Oropharyngeal Swab, Sputum, Respiratory secretion, etc.

2. Procedure:

The performance of the SN CoV Ensure One Step Multiplex SARS CoV-2 RT-PCR Detection Kit depends on the amount and quality of template RNA purified from the clinical samples.



The extraction protocol should be performed following manufacturer's instructions or an internally validated protocol.

3. SN CoV Ensures PCR Master Mix preparation:

1. Prepare the master mix as per table mentioned below. Positive control (PC) and Negative test control (NC/NTC) should be included in every set up.

Color Code	Components	Volume (μL)/Reaction	Volume (μL) for 100 Reaction
Natural	SN CoV Ensure RT Master Mix (MM)	10 μL	1000 μL
Amber Tube	PP Mix	2 μL	200 μL
Total Volume		12 μL	1200 μL

Add each component to multiple number of samples to be processed and prepare the master mix. It is advisable to prepare extra mix for one sample for any pipetting related error or dead volumes.

Note:

1. Dispense 12 μL from above master mix in separate tubes/wells
2. Add 8 μL of isolated RNA from patients sample separately to each tube

Once dispensed, seal the plate with optically clear sealers or close the tubes-strips, centrifuge the tubes/strips or plates briefly and place them in the instrument.

4. Thermal Profile: (Setting of RT-PCR)

Referring to the instrument manual, set on the dedicated software for the parameters of thermal cycle.

Steps	Temp	Time	Cycle
Hold 1	50 °C	15 min	1
Hold 2	95 °C	2 min	1
Cycling	95 °C	15 secs	40
	60 °C	30 secs	

5. Fluorescence Setup:

Target	Applied Biosystem 7500	BioRad CFX-96	Rotor Gene Q
E gene	Cy5	Cy5- RdRp gene	Red Channel
RdRp gene	FAM	FAM-E gene	Blue Channel
RNase P	VIC	HEX- RNase P gene	Green Channel

Note:

The Kit is compatible with; Rotor Gene™ 3000/6000, ABI Step One, 7500/FAST, Quant studio, BioRad CFX 96, Roche 480 and other Real Time PCR machine available.

6. Precautions for PCR:

The following aspects should always be taken care of:

- a) Store positive material (Specimens, Positive controls & amplicons) separately from all other reagents and add it to the reaction mix in a separate area.
- b) Thaw all components thoroughly at room temperature before starting the assay.
- c) When thawed, mix the components and centrifuge briefly.
- d) Work quickly on ice or in the Cooling Block.
- e) All the reagents including the NTC (except for PCs & specimens) should be mixed & dispensed in pre-mix area.
- f) All the controls & specimens should be mixed & dispensed in extraction area.
- g) Use pipette tips with filters only.
- h) Always use disposable powder-free gloves.
- i) Discard samples and assay waste according to your local safety regulations.
- j) Do not open the reaction tubes or plates post amplification.

7. Additionally, required Materials and Devices

- I. Appropriate 3 or more channels RT-PCR instrument, compatible with the fluorophores used in this test.
- II. Appropriate nucleic acid extraction system or kit. It is recommended to use commercialized extraction kit extracting RNA.
- III. Vortex mixer.
- IV. Centrifuge with a rotor for 2 mL micro centrifuge tubes.
- V. Pipette tips with filters (disposable).
- VI. Powder-free gloves (disposable).
- VII. Class II Biological Safety Cabinet (BSC), ideally in a BSL-2 facility.

8. Result Interpretation:

Amplification of PC suggest absence of any inhibition during amplification. Amplification of RNase P in samples suggest proper RNA extraction. Amplification in Cy5 and FAM channels indicate presence of SARS Cov-2 in sample. Due to competition and limiting factors, samples with high viral copy may not show amplification in VIC (IC) channel and the curves may not be a proper sigmoid. Results can be analyzed with the help as mentioned below:

9. Data analysis:

Ct cut off value for Cy5 and FAM is ≤ 34 , Sigmoidal curves.

Note:

Ct value in any sample/well > 34 should be considered as below detection limit of the kit



10. Result Interpretation table:

SL.NO	HEX (internal control) RNase P	CY5 RdRp	FAM E gene	Result Interpretation
1.	+/-	+	+	Novel corona virus SARS Cov-2 RNA detected
2.	+/-	+	-	Novel corona virus SARS Cov-2 RNA Not detected
3.	+/-	-	+	Novel corona virus SARS Cov-2 RNA detected*
4.	+	-	-	All targets are valid, SARS CoV-2 RNA NOT detected
5	-	-	-	Invalid repeat sampling/extraction/amplification

* Retest the sample if the retest results are same, then it could be presumed as positive.

11. Analytical Performance:

Evaluation of the kit on the confirm positives and negative specimen showed 100% specificity and sensitivity. The kit can detect 1×10^3 copies per ml of SARS CoV-2 RNA from clinical sample.

12. Trouble shooting:

Problem Possible Cause Recommendation		
	Error in Master mix preparation	Check the dispensing volume during preparation of master mixture
No signal in all samples including positive control	Inhibitors added Probe degradation Positive control degradation Omitted components Instrument setting error	Repeat the extraction step with new sample, use a new probe reagent, use a new positive control Verify each component, repeat the RT-PCR mixture preparation Check the position setting for the positive control on the instruments. Check the Thermal cycle settings on sample instrument
Diverse intensity of fluorescent signals	Pipetting error Contamination in the outer surface of PCR tubes or Plate	Make sure that the equal volume of reactants is added in each tube or plate. Wear gloves during the experiment
Weak or no fluorescent signal in samples only	Poor RNA quality, Insufficient volume of RNA	Use recommended kit for RNA extraction and store extracted RNA at -80°C, Repeat PCR reaction with correct volume of RNA Fluorescence



No template Control (NTC) showing Amplification	Amplification of SARS CoV-2 genes in a no Template Control indicates contamination in one or more of the reagents, incorrect placement of a plate or tubes into the RT-PCR instrument, or pipetting error	Repeat the test by using 2 NTC. If NTC fails again, then investigation should be conducted to identify possible causes of error
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13. Technical Assistance:

For technical assistance please contact our technical Support: Email: drmondal@snlifesciences.com
Phone: +91 8095961944

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