Product Name : Isopollo® COVID-19 detection kit (real-time)

Cat No. : 52227

Packing Unit : 100 tests / kit **Storage :** -25 ~ -15℃

Expiry Date : 6 months from date of manufacture

For in vitro diagnostic use only

【Description】

Isopollo® COVID-19 detection kit (real-time) is an *in vitro* diagnostic kit for qualitative analysis to diagnose severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection from extracted RNA of clinical specimens of smeared material from human nasopharyngeal and oropharyngeal swab, sputum, and bronchoalveolar lavage by RT-LAMP (Reverse transcription loop-mediated isothermal amplification) and it has high specificity for targeted RNA because 6 primers selectively detect specific genes (RdRP gene and N gene) of SARS-CoV-2. The results can be quickly checked in real-time without such electrophoresis etc.

【Intended Use】

Isopollo® COVID-19 detection kit (real-time) is *in vitro* diagnostic reagent kit for qualitative analysis to detect SARS-CoV-2 infection from extracted RNA of clinical specimens of smeared material from human nasopharyngeal and oropharyngeal swab, sputum, and bronchoalveolar lavage of patients suspected SARS-CoV-2 by RT-LAMP.

【Characteristics】

This product has some special characteristics as follows :

⋆ High specificity : Due to using 6 distinct primers for the specific sequence, the kit shows high specificity than conventional PCR which uses 2 primers for the sequence.

⋆ High efficiency : Reaction time is quite shorter than conventional real-time PCR as 30 mins or less, since not needing cyclic thermal change during the whole procedure. At the same time, DNA or RNA loss reduces with isothermal environment, which enables highly efficient amplification of nucleic acids.

【Contents of the kit and Quantity】

Reagents	Vol.	Quantity	Retention period
2X Reaction Buffer	1250 ㎕	2	6 months
Enzyme mix	200 ㎕	1	
Detection primer (CR)	200 ㎕	1	
Detection primer (CN)	200 ㎕	1	
Control primer*	40 ㎕	1	
Control template*	40 ㎕	1	
Distilled water (DW)	1.5 ㎖	1	

* Control primer & Control template contained for 20 reactions

[Protocol]

1. Sample preparation and nucleic acid extraction

① Smeared specimen collected from human pharynx suspected of having SARS-CoV-2 using a swab is used. If a swab is used, it is recommended to use samples collected by rubbing mucous membrane from nasal cavity through to the laryngopharynx.

② The collected samples should be used immediately or stored at -20℃.

③ Nucleic acid should be isolated using useful viral RNA extraction kit according to the manufacturer's instructions.

2. Reagents preparation

① Take out the reagents stored at -20℃, and thaw them at room temperature. Once the reagents are thawed, keep them on ice.

② Prepare 25 ㎕ LAMP reaction mixture as follows:

- Check for RdRP gene

Reagents	Volume (1 reaction)
2X Reaction Buffer	12.5 ㎕
Enzyme mix	1.0 ㎕
Detection primer (CR)	2.0 ㎕
Extracted RNA (Template)	5.0 ~ 9.5 ㎕
Distilled water*	- ㎕
Total	25.0 ㎕

* If the number of samples to be examined is so many, it is recommended that the mixture be calculated according to the number of responses and used in a 1.5 ml tube.
* In case of distilled water, adjust and add according to the template volume.
* For control reactions, use 2 µL of Control template, 2 µL of Control primer, and 7.5 µL of DW as positive control and use 9.5 µL of DW instead of RNA as negative control.

- Check for N gene

Reagents	Volume (1 reaction)
2X Reaction Buffer	12.5 ㎕
Enzyme mix	1.0 ㎕
Detection primer (CN)	2.0 ㎕
Extracted RNA (Template)	5.0 ~ 9.5 ㎕
Distilled water*	- ㎕
Total	25.0 ㎕

* If the number of samples to be examined is so many, it is recommended that the mixture be calculated according to the number of responses and used in a 1.5 ml tube.
* In case of distilled water, adjust and add according to the template volume.
* For control reactions, use 2 µL of Control template, 2 µL of Control primer, and 7.5 µL of DW as positive control and use 9.5 µL of DW instead of RNA as negative control.

3. Operation procedure

① Select wavelength of FAM or SYBR Green 1 in real-time PCR machine (CFX96™ Dx System, Bio-Rad, CA, USA) or equivalent (Applied Biosystems™ 7500 Real-time PCR Instrument system, Applied Biosystems™ 7500 Fast Real-time PCR Instrument system, Thermo Fisher Scientific, MA, USA). And perform the reaction as follows:

Step	Temperature	Time	Cycles
1	58°C	30 sec	40
2	80°C	2 min	1

4. Detection

- ⋆ Positive criterion Ct value : within 40 Ct
- ⋆ Negative criterion Ct value : No detection
- ⋆ Interpretation of results

CR	CN	NC	Positive /Negative	Interpretation
+	-	-	Positive	RdRP gene detected
-	+	-	Positive	N gene detected
+	+	-	Positive	RdRP gene and N gene detected
-	-	-	Negative	SARS-CoV-2 not detected
( )	( )	+	Invalid	Invalid and recommend re-examination

【Warnings and Precautions】

1. When handling the samples, always comply with the rules for biohazard regulation to prevent infections from unknown microbes or diseases. After finishing experiment, dispose the laboratory wastes considering as biological wastes.
2. This kit is very sensitive. Thus, it can be easily contaminated by own amplified products. If conducting electrophoresis (usually not necessary for our protocol), highly cautious attention is needed especially when opening the cap of final reaction tubes.
3. Since RNA is vulnerable to RNase, careful handling is essential during the procedure. There is always chance for contamination of RNase from such as sample specimens (blood, urine, tissue, etc.), experimental tools, reagents, water, and the operator's saliva or perspiration. RNase is stable against heat and might maintain its activity even after autoclave sterilization. In order to avoid RNase contamination, the following points should be concerned.
   - Designate exclusive operation area and tool for RNA treatment.
   - Use sterilized disposable tubes and pipette tips.
   - Use RNase free water (for example, DEPC treated water).
   - The operator must wear gloves and masks to prevent RNase contamination to the specimen from his or her saliva and perspiration.
4. This kit should be stored at -25℃ ~ -15℃. We recommend to take out the necessary amount of reagents from the freezer before use to prevent deterioration of the reagents. Do not repeat unnecessary freezing and thawing. When thawing the reagents, remain them at room temperature a while, after thawing keep them on ice for preparation step. If storing for a long time, keep the storage temperature much lower as -80℃.
5. Before the reaction, mix the solution in PCR tubes well and then spin down the tubes to drop down the solution staying on the tube wall or on the cap. Notice that fierce mixing should be avoided as it can inactivate the enzyme.
6. Since bubbles in the solution will interfere the exact judgment, try not to cause any bubble when mixing the solution. If bubbles are present, spin down to get rid of the bubbles.
7. Keep the cap of the used tube completely closed and dispose it, according to the relevant regulations and instructions, by incineration or after double bagging it with sealable vinyl bag.
8. This kit is designed for *in vitro* diagnostic use only.

[Descriptions of Symbol marks]

REF Catalogue number
IVD *In Vitro* Diagnostic
Σ XX Sufficient for XX tests
Storage temperature limitation