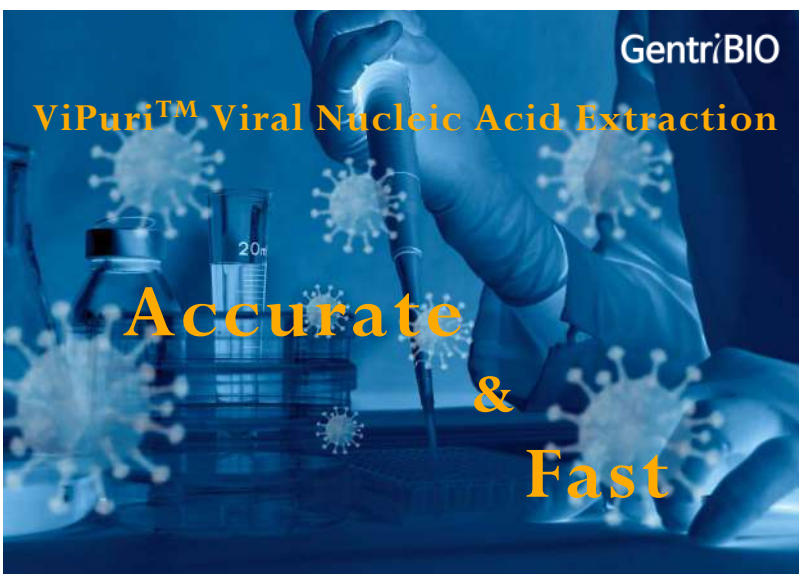




### Excellent Choice for Coronavirus RT-PCR Detection

Here, we have simulated the most challenging viral RNA isolation cases by various concentrations of virus particles which represent different stages of viral infection in cell-free or non-cell free conditions using the **ViPuri™ Viral RNA Purification Kit** and One-Step RT-PCR Master Mix (Fig. 1, 2 & 3).



Generally, the genomes of RNA viruses are protected by outer layers of lipid membranes or protein capsids. The outer layer of coronavirus is composed of the lipid membrane and spike proteins. Coronavirus also has the inner capsids, called nucleocapsid however, do not hold integrity comparable to outer protein capsid. Therefore, any condition that capable break eukaryotic cell membranes can also break the coronavirus outer layer in contrast to the protein capsids. For example, the protein capsids from norovirus are much more rigid therefore, harder to break compares to the enveloped virus, the lipid membraned virus. Therefore, it used as a test virus to verify the overall performance of the ViPuri™ Viral RNA Extraction System in cell free or non-cell free condition.



Fig. 1. ViPuri™ Viral RNA Purification Kit

Practically, most of nasopharyngeal (NP) / oropharyngeal (OP) swabs from coronavirus infected patients contain the highly keratinized epithelial cells, mucus, many different enzymes and immune cells, not only the free virus particles and viral genomes inside the infected cells.

Therefore, the kit should be able to lysis the lipid membranes including cell membrane and viral membrane but also tough protein viral capsid as well. It is often the case that viral RNA extraction kits were not able to handle various sample composition or clogged the column giving false-negative results.

The ViPuri™ Viral RNA Purification Kit could be accurately isolated viral RNA from coronavirus infected patients as explained in the results below. This fact suggest that it is very useful tool in the process of viral infection diagnosis.



Fig. 2. Detection of the viral RNAs from an OP swab samples with mixed various amounts of +ssRNA virus (Norovirus) protected by rigid protein capsid. Single OP swab using Q-tip from healthy volunteers were dropped into 2 mL of PBS buffers with various virus particles. To isolate viral RNAs, 150  $\mu$ L of the sample was processed with each kit then, 1  $\mu$ L of final viral RNA elution was used for RT-PCR detection. The RT-PCR was performed using the One-step RT-PCR Master Mix with following conditions: reverse transcription at 50°C for 15 min; RT inactivation at 95°C for 2 min; 35 cycles of the following steps: 95°C for 15 sec, and 60°C for 30 sec.

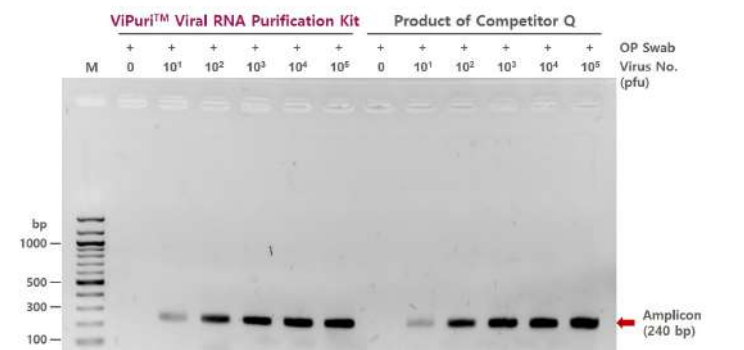


Fig. 3. Comparison results of the viral RNA extraction ability between ViPuri™ Viral RNA Purification Kit of GentriBIO and the product of competitor Q.

By the detection experiment, the ViPuri™ Viral RNA Purification Kit verified that the Limit of Detection (LoD) was the 7 virus particles (100 virus particles in 2 mL of PBS buffer).

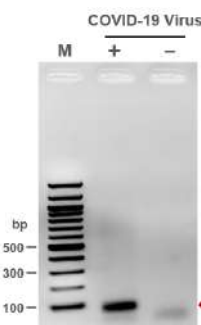


Fig. 4. Detection of COVID-19 virus' N-gene from the saliva of coronavirus infected patient using the ViPuri™ Viral RNA Purification Kit. RT-PCR experiment was performed with the 1  $\mu$ L of isolated RNA and One-Step RT-PCR master mix with following conditions: Reverse Transcription at 50°C for 15 min; Inactivation of reverse transcription at 95°C for 2 min; PCR for 35 cycles of the following steps: 95°C for 15 sec, and 60°C for 30 sec. To detect whether COVID-19 virus infected or not, we used the primer set as like US CDC 2019-nCoV\_N1 (72 bp).

Finally, we are suggest that the ViPuri™ Viral RNA Purification Kit could be effectively applied to virus infection diagnosis with its ability to extract viral RNAs accurately and quickly even in COVID-19 virus infected patients.