*RNA*GEM V Quick-Start-Guide: Virus

This Quick-Start-Guide is for extracting RNA from Virus using the *RNA*GEM V kit - *for RNA extraction from Cell Culture, please see the RNAGEM kit*

*RNA*GEM V Principle

*RNA*GEM V utilises a novel thermostable protease and buffer system to extract RT-PCR-ready nucleic acids from viruses collected on swabs and/or stored in viral storage buffer (see important notes section) as well as saliva samples.

*RNA*GEM V does not use traditional proteinase K methods, meaning RNA extractions can be carried out in a single tube, without the need for multiple wash steps.

This ensures high RNA recovery and a low risk of contamination.

Extractions can be performed in a standard thermocycler and RNA extracted with *RNA*GEM V is compatible with any PCR system.

When your kit arrives

New RNAGEM V reagents are stable at room temperature. After the reagent tubes have been opened they should be stored at -20°C to ensure enzyme stability.

Important Notes

With *RNA*GEM V not having purification steps such as magnetic beads or spin column etc. any RT-PCR inhibitors that are loaded into the extraction will be present after extraction and can therefore interfere with downstream analysis. The cleaner the sample used, the cleaner the final extract.

When working with storage buffers it is important to work with buffers that do not contain compounds that could interfere with downstream analysis. We recommend validating the method with your chosen storage buffer to determine compatibility. A series dilution of the starting sample or final extract can help to identify an ideal volume to limit the amount of inhibitors added to downstream analysis.

***Important: *RNA*GEM V has been shown to be compatible with swabs collected in 2 ml saline solution (0.9%NaCl) and PBS. Lower volumes can lead to inhibition of the RT-qPCR, producing delay in Ct values. In addition, RNAGEM V is also compatible with some UTM media. Incompatible UTM media may lead to Ct value delays**

Expected Results

Total Nucleic Acid suitable for	RT-PCR & RT-qPCR
Final Volume	~ 100µl
Recommend amount of RNA template for RT-qPCR	1-5µl in a 25µl RT-PCR
Quantification Method	RT-qPCR, Fluorometric Assay

Method

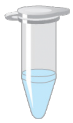
1a. For RNA extraction from viral storage buffer such as PBS/Saline solution (0.9% NaCl) or UTM*, vortex the buffer and pipette 89µl of buffer into a new micro-centrifuge tube.

1b. For RNA extraction from saliva samples, pipette 45µl of the saliva sample and 44µl of nuclease-free water into a new micro-centrifuge tube.

2. Pipette the following into the same tube from step 1:
- 1µl RNAGEM
- 10µl 10x Blue buffer

Briefly vortex and spin to mix. Total extraction volume should be 100µl

3. Place the tube into a thermal cycler and incubate at:
- 75°C for 10 minutes
- 95°C for 5 minutes



This solution now contains DNA and RNA (Total Nucleic Acid) ready for RT-PCR and RT-qPCR

Technical tips and suggestions

- *RNA*GEM V is a preparative method for RNA extraction. The method lyses cells and removes nucleoproteins to extract RNA
- There is no concentration step in the procedure and so the concentration of the extract is dependent on the quality of the sample and the extraction volume used.
- Protocols can be scaled up/down according to your concentration requirements.
- *RNA*GEM V extracts are complex lysates containing nucleic acids and peptides. For this reason, OD_{260} methods for yield estimation, such as Nanodrop and UV-spectrophotometry, are not-suitable for quantifying RNAGEM V extracts.
- For accurate yield assessment, florescence methods such as RT-qPCR, Qubit, Pico Green etc. are recommended.
- *RNA*GEM reagents, once mixed, should be run within one hour. For extended periods, reagent mixes should be kept frozen at -20°C.
- Always use certified DNA-free water, tubes and reagents.
- RNAGEM is sensitive to EDTA and other chelating agents
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- For long term storage of the extracted RNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C.