

**Before using the Galenvs viral extraction kit, it is important to add ethanol (>95%) to wash buffers 1 & 2 as per label instructions. It is recommended to mark the bottle top label to indicate ethanol has been added.*

**Before each extraction experiment, make sure that all bottles are well mixed by inverting upside down several times.*

****Caution!!!** The magnetic rack contains a very powerful magnet that could cause physical harm if not handled carefully. Also, avoid placing the magnet near electronic equipment such as computers and cellphones.*

1) How many microfuge tubes should be used for each extraction?

- The whole extraction protocol should be performed in a single tube. It is recommended to transfer the clean RNA eluate to a new tube for storage or downstream experiments.

2) How many extractions should be performed using magnetic racks?

- Each extraction in a single tube can be performed in 15-20 minutes and needs a single magnetic rack. Many extractions can be performed if using several magnetic racks in parallel. However, it is recommended to perform 8 extractions at a time with 8 racks as increasing the number of extractions can lead to unwanted user error.

3) Can any commercial magnetic rack be used?

- Yes, any commercially available magnetic rack will be compatible. However, depending on the magnet strength, capture time may vary.

4) What sample types can be used for viral RNA extraction?

- Any cell-free media can be used for viral RNA extraction. This includes swab preservation solution, viral inactivation media, viral transport media, cell culture supernatants (ie. DMEM or other media), saliva, or urine.

5) When should carrier RNA be used?

- It is recommended to use carrier RNA if the viral load is very low. Galenvs recommends using carrier RNA if the viral copy number is below 100 copies/ μ L of sample.

6) Can water be used for elution?

- Nuclease-free water or DEPC-treated water may be used for elution.

7) Can elution volume be more or less than 50µL?

- 50uL is the recommended volume as it allows for an appropriate concentration of RNA for downstream processing (e.g. PCR, NGS, etc). However, less volume (20-50uL) can be used to obtain a more concentrated sample. Also, more volume (up to 200uL) can be used to obtain more dilute eluate if needed.

8) Is heating or vortexing necessary for viral RNA extraction?

- No heating or vortexing is required for the Galenvs viral RNA extraction protocol

9) Can vortexing be used for mixing instead of pipetting up/down?

- It is recommended to mix by pipetting since vortexing may cause the sample to be trapped under the lid of the microfuge tube. This, in turn, will require a short spin/centrifugation to retrieve and collect all the samples.

10) Which pipette tip sizes should be used?

- For carrier RNA addition, 1-10uL tips should be used; For sample, 10-100uL or 200uL tips should be used; For lysis/binding and wash buffers, 100-1000uL tips should be used; For elution, 10-100 or 200uL tips should be used.

11) After discarding supernatant following wash #2 and before elution, there is still liquid remaining. Is 1 minute enough to dry beads before elution?

- It is important to try to discard all remaining solution following wash #2. Usually, 1 min drying should be enough but can be increased to 3 minutes if necessary. Do not leave to dry for over 5 minutes as the beads become too dry – some wetness is still necessary for efficient elution.

12) Why should the capture for Wash #1 be for 5 minutes and only 1-2 minutes following Wash #2?

- We recommend incubation on the magnetic rack following wash #1 of 5 minutes to ensure that any contaminants will be desorbed from the bead surface. This is not necessary for wash #2, as the beads have become cleaner and the only removal of salts is required.