# rNEAT™ Viral RNA Extraction Kit

PURK-RNV-100

## Description

The rNEAT™ Viral RNA Extraction Kit is designed for the rapid purification of viral RNA from cell –free samples such as serum, plasma, urine, cell free body fluids, cell culture supernatants and rinse liquid from swabs samples. The viral RNA molecules bind to the silica-based media and impurities are removed by thorough washing with Wash Buffer. The RNA is then eluted in sterile, RNase free water. The isolated viral RNA is ready to use and should be stored at - 70°C. The procedure can be used for isolation of viral RNA from a broad range of viruses. However, performance cannot be guaranteed for every virus species and must be validated by the costumer. The amount of purified viral RNA depends on the sample type, the viral titer, sample source, transport, storage, and age. The Kit also includes carrier RNA that improves binding and recovery of low-concentrated viral RNA.

## Applications

The purified viral RNA is suitable for use in RT-PCR and qRT-PCR and can be used for:

- ✓ Viral load monitoring
- ✓ Viral detection
- ✓ Viral genotyping

| Kit Components | 50 rxn |
|---|---|
| Buffer BLY* | 40 ml |
| WB1 Buffer** | 33 ml |
| WB2 Buffer** | 20 ml |
| RNase-free water | 10 ml |
| Mini-spin column | 100 |
| Collection tubes 2 ml | 200 |
| Carrier RNA (Lyoph.) | 1,6 mg |

## Storage

Store the Carrier RNA (Lyophilized) at –20 °C and all other components at room temperature (18 to 25 °C). If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and allow cooling to room temperature before use.

*Add appropriate volume RNase-free Water (included with the kit) to lyophilized Carrier RNA to obtain 4 µg/µL Carrier RNA stock solution. Prepare aliquots of 100 µl and store -20ºC. For 25 purifications, thaw one vial of 100 µl Carrier RNA and mix thoroughly with 10 ml BLY-Buffer. Mark the label of the bottle to indicate that Carrier RNA was added. Carrier RNA has a limited shelf-life in BLY-Buffer.

**Add the volume ethanol (96%-100%) specified [Not included] to WB1 and WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

## Quality Certifications

The quality of the kit is tested on a lot-to-lot basis by isolating viral RNA from a 200µl serum sample.

## Product use limitation

This product is developed, designed, and sold exclusively for research purposes and use only. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals

# Protocol

1. Transfer 200 µL of sample (plasma, serum, urine, body fluids or cell cultured supernatant) into a microcentrifuge tube (not provided).
   - **Nasopharyngeal swab (NP) /oropharyngeal swab (OP)**: If the swab is delivered in a transport media suitable for nucleic acid virus stabilization, transfer 200 µl directly into microcentrifuge tube. If you get a swab without transport media, place the swab into microcentrifuge tube containing PBS and incubate for 15 minutes at RT. Afterwards shake the swab vigorously, squeeze it and remove the swab. Use a 200 µl aliquot of the liquid for viral RNA extraction.
2. Add 400 µL BLY-Buffer (containing Carrier RNA) and mix immediately.
   - **Carrier RNA** enhances binding of viral RNA to the silica membrane and reduces the risk of viral RNA degradation.
   - **Internal Extraction Control**: When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer (BLY-Buffer). This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. DO NOT add the internal control RNA directly to the biological sample as this will lead to degradation and a loss in signal strength.
   - **Proteinase K treatment**: Although Proteinase K treatment is usually not required for viral RNA isolation, it is recommended for isolation from viscous samples (e.g., sputum samples). Add 25µL Proteinase K (20 mg/ml stock solution), to the lysis mixture and vortex vigorously for 20 seconds. Incubate for 5 min at 70ºC. [Proteinase K not included in the kit].
3. Incubate the mix at RT for 10 minutes.
4. Place the minispin-column in a 2 ml Collection tube and transfer the lysed sample from previous step. Centrifuge at 8000xg for 1 minute. Discard the flow-through.
   - For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and nonviscous sample before loading onto the minispin- Column.
5. Place the minispin-column in a new Collection tube and add 500 µL of WB1 Buffer. Centrifuge at 8000xg for 1 minute. Discard the flow-through.
6. Place the minispin-column in the same Collection tube and add 500 µL of WB2 Buffer. Centrifuge at 8000xg for 1 minute. Discard the flow-through.
7. Place the minispin-column in the same Collection tube and add 500 µL of WB2 Buffer. Centrifuge at 8000xg for 1 minute. Discard the flow-through.
8. Centrifuge at full speed for an additional 3 min to dry the minispin-column.
   - This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
9. Place the minispin-column into a new, labelled 1.5 microcentrifuge tube (not provided) and pipet 50 µL RNase-free Water directly into the membrane. Close the cap and incubate for 2 minutes at RT.
10. Centrifuge at full speed for 1 minute to elute. The eluate contains viral RNA. After extraction place the Elution Tube on ice. For long time storage place the nucleic acids at –80°C.
    - Final eluates contain viral RNA and Carrier RNA; therefore, it is not possible to quantify the nucleic acids isolated with the kit by photometric or fluorometric methods.