Viral RNA extraction Kit

(500 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22101-500

www.zellx.de

For use with standard and fast qPCR platforms

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Background

ZellX® Viral RNA extraction Kit is designed for the quick purification of viral RNA from cell-free samples such as serum, plasma, urine, and other body fluids, cell culture supernatants and rinse liquid from swabs samples.

The viral RNA molecules bind to the silica-based membrane, and impurities such as proteins and nucleases 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 quantity of purified viral RNA depends on different parameters including the sample type, virus titer, sample source, transport, storage, and age. Our Kit additionally contains carrier RNA that improves binding and recovery of low-concentrated viral RNA.

Intended use

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

Viral detection | Viral load monitoring | Viral genotyping

Materials supplied in the Kit

Component	*Quantity*
Minispin Columns	500
Collection tubes	1000
RNase free water	50 mL
Wash Buffer 1	165 mL
Wash Buffer 2	100 mL
Lysing Buffer	300 mL
Carrier RNA	1 vial

Storage instruction

All reagents should be stored at room temperature except the vial of lyophilized Carrier RNA which must store at -20°C upon receipt. Avoid repeated freezing and thawing.

Materials required but not supplied

Biological Safety Cabinet & Personal Protective Equipment (according to local guidelines for working with potentially infectious material in particular if material is derived from a human or animal sample).

Precision pipettes and disposable filter pipette tips (RNase & DNase free)

Vortex and micro-centrifuge (10000 g)

Sterile 1.5 mL micro-centrifuge tubes (RNAse-free)

Ethanol 96%-100% (we recommend BioUltra for molecular biology, from Sigma-Aldrich Cat. No. 51976)

Proteinse K (Optional for viscous samples)

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- If any kit reagent forms a precipitate, warm it up at 55-65 °C until the precipitate dissolves, and allow cooling to room temperature before use.
- This kit can be used for automated RNA isolation but it is neither compatible with magnetic-based robotic workstations nor with microplate-based robotic workstations

Reagent preparation

i. **Carrier RNA:** Resuspend supplied lyophilized vial of Carrier RNA in 3 mL of supplied RNAse-free Water and mix thoroughly. Aliquot it for weekly use based on provided table (below) and store them at -80 °C for up to 6 months. **More than 3 freeze-thaw cycles of Carrier RNA must be avoided**.

No. of RNA sample	*Carrier RNA*	*LYSING Buffer*
12	72 µL	7.2 mL
24	144 µL	14.4 mL
48	288 µL	28.8 mL
96	570 µL	57 mL
192	1.15 mL	115 mL
384	2.3 mL	230 mL
480	2.88 mL	288 mL
500	3 mL	300 mL

Wash Buffer 1&2: Add the appropriate amount of molecular biology grade ethanol (96%-100%) [Not provided] to Wash Buffer 1 and Wash Buffer 2 prior to initial use (see bottle label for volume).

Note: Although for isolation of viral RNA, Proteinase K (not included) treatment is usually not required, 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 mix by vortexing vigorously for 5 seconds. Incubate for 5 minutes at 70°C. Alternatively, if the sample is very viscous and there is no further reaction of isolated RNA, then a more diluted sample may be used for a new RNA isolation, for example a 1/2 to 1/10 of starting sample may be used.

Sample preparation

- ✓ **Nasopharyngeal swab (NP) /oropharyngeal swab (OP):** If the swab is delivered in a transport media suitable for nucleic acid virus stabilization, transfer 140 µL directly into a microcentrifuge tube. If it is handeled without transport media, place the swab into a microcentrifuge tube containing PBS and incubate for 15 minutes at room temperature. Afterwards shake the swab vigorously, squeeze it against the wall of tube and remove the swab. Use a 140 µL aliquot of the liquid for viral RNA extraction.

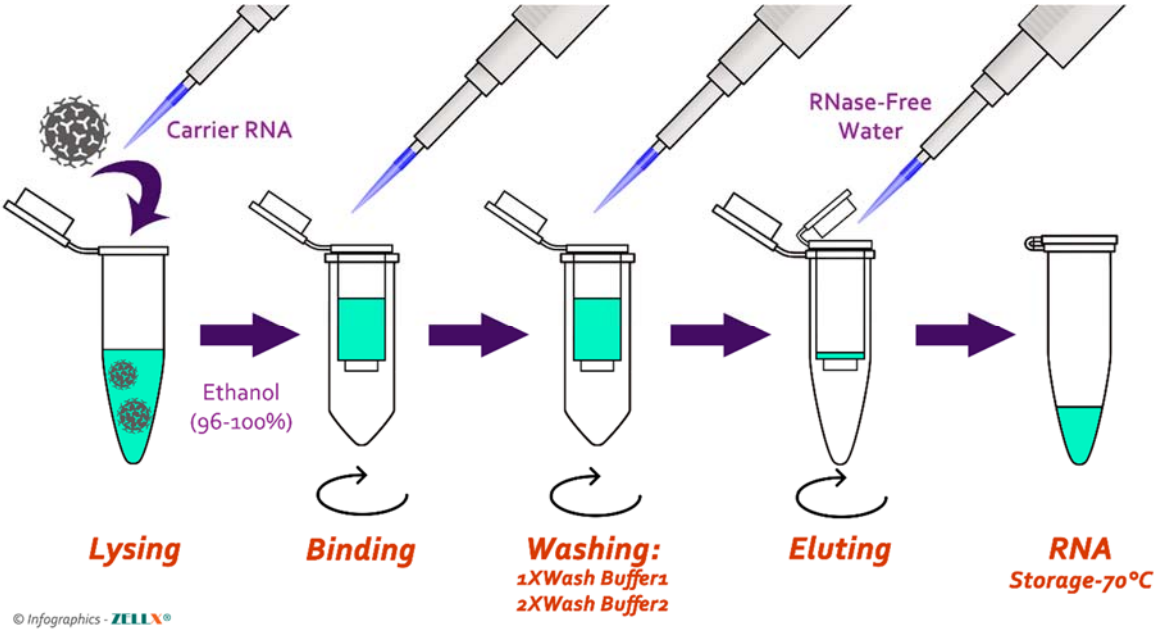
- ✓ **Internal Extraction Control:** When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the Lysing buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process.

- Do not add the internal control RNA directly to the biological sample as this will lead to degradation and a loss in signal strength.

Assay Procedure

1. Transfer 560 µL of Lysing Buffer (containing Carrier RNA) into a 1.5 mL microcentrifuge tube. (Carrier RNA enhances binding of viral RNA to the silica membrane and reduces the risk of viral RNA degradation).
2. Add 140 µL of sample (plasma, serum, urine, body fluids or cell cultured supernatant) and mix by vortexing for 5 seconds.
3. Incubate for 10 minutes at room temperature (RT).
4. Add 560 µL of ethanol (96-100%) to the sample, and vortex for 5 seconds.
5. Place a Minispin-column in a 2 mL Collection tube and transfer 700 µL of the lysed sample into the Minispin-column. Centrifuge at least at 10000 g for 1 minute. We recommend to centrifuge at maximal speed, and discard the flow-through.
6. Repeat the step 5 until all the sample has been transferred to the Minispin-column.
7. Place the Minispin-column in a new Collection tube and add 500 µL of Wash Buffer 1. Centrifuge at least at 10000 g (or maximal speed) for 1 minute. Discard the flow-through.
8. Repeat the washing step (step 7) two more times, using 500 µL of Wash Buffer 2.
9. Centrifuge at full speed for an additional 3 minutes to dry the Minispin-column. Rotate the column 180° and repeat centrifugation at full speed for 3 more minutes to dry the Minispin column.
10. Place the Minispin-column into a new, labelled 1.5 microcentrifuge tube (not provided) and pipet 50 µL of RNase-free Water directly onto the membrane (The tip should not touch the membrane). Close the cap and incubate for 2 minutes at RT.
11. Centrifuge at maximum speed for 1 minute to elute. The eluate contains viral RNA. After extraction place the Elution Tube on ice. For long time storage keep it at -80°C.

- As the final eluates contain viral RNA and Carrier RNA, it is not possible to quantify the nucleic acids isolated with the kit by photometric or fluorometric methods.


Carrier RNA
Ethanol
(96-100%)
RNase-Free
Water
Lysing
Binding
Washing:
1XWash Buffer1
2XWash Buffer2
Eluting
RNA
Storage-70°C
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