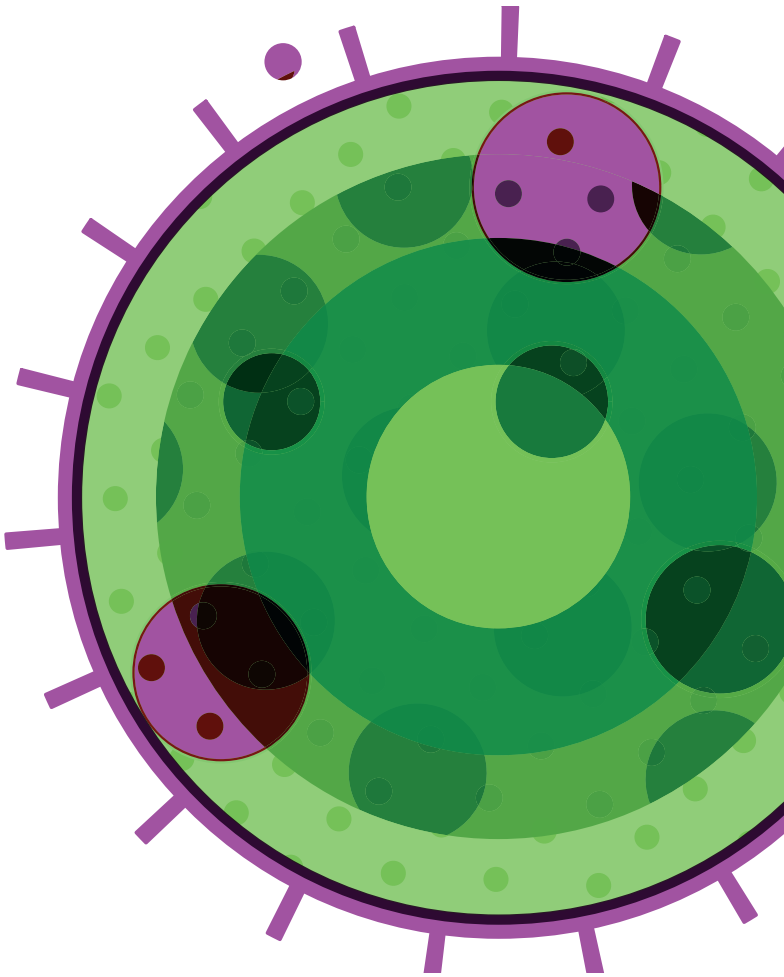
EXTRACTME®
VIRAL RNA KIT

Cat. No. EM39
Version: 1.2020

Kit for viral RNA isolation from swabs



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I. INTENDED USE

The ***EXTRACTME*** **VIRAL RNA KIT** is designed for the rapid and efficient purification of high quality viral RNA from swabs. The kit is specifically designed to isolate viral nucleic acid from a variety of RNA viruses including SARS-CoV-2 (the virus that causes COVID-19). The isolation protocol and buffer formulation were optimized for high isolation efficiency and RNA purity. The product is intended for research use only.

II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS	Storage Conditions[1]
Catalogue number	EM39-010	EM39-050	EM39-250	
⚠ **vRLys Buffer** (viral RNA Lysis Buffer)	4 ml	20 ml	100 ml	RT in dark
vRW Buffer* (viral RNA Wash Buffer)	4 ml	19 ml	90 ml	RT
vREB (viral RNA Elution Buffer)	1 ml	7.5 ml	7.5 ml	RT
RNA Purification Columns placed in Collection Tubes	10 pcs	50 pcs	5 x 50 pcs	RT

[1] RT – room temperature (+15°C to +25°C)

* Prior to the first use add an appropriate amount of **isopropanol** to the **vRW Buffer**; for details, see the instructions on the bottle label as well as in the table below. Marking the bottle with added alcohol is recommended.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM39-010	EM39-050	EM39-250
vRW Buffer*	4 ml	19 ml	90 ml
isopropanol	16 ml	76 ml	360 ml
Total volume	20 ml	95 ml	450 ml

* The diluted vRW buffer might be prepared in a smaller volume than given in the table and it should be diluted as follows: 1 volume of vRW Buffer to 4 volumes of alcohol. E. g. for 10 isolations use 4 ml vRW Buffer concentrate and 16 ml isopropanol.

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

⚠ **Protect the vRLys Buffer from the sunlight!**

Expiry date

Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- isopropanol
- 1.5–2 ml RNase-free microcentrifuge tubes
- automatic pipettes and pipette tips (RNase-free)
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 12 000 x g)
- vortex mixer
- freezing rack

IV. PRINCIPLE

The ***EXTRACTME*** **VIRAL RNA KIT** utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids at high concentration of chaotropic salts. During the first isolation step, the material is lysed under highly denaturing conditions to inactivate nucleases and to ensure isolation of intact viral RNA. A homogenate is lysed with guanidine thiocyanate and detergents. RNases are inactivated by guanidine thiocyanate. RNA bounds to a Purification Column membrane by addition of alcohol. A three-step washing stage effectively removes impurities and enzyme inhibitors. Purified RNA is eluted with the use of low ionic strength buffer and may be used directly in all downstream applications, such as RT-PCR, RT-qPCR, cDNA synthesis.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **VIRAL RNA KIT** is tested with the use of standard QC procedures. Purified RNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL
swab in viral transport medium (e.g. nasopharyngeal swabs, mouth and throat swabs)
EFFICIENCY
dependent on the sample material amount and type
BINDING CAPACITY
~120 µg RNA
TIME REQUIRED
10–12 minutes
RNA PURITY
A_{260}/A_{280} ratio = 1.9 – 2.1

VII. SAFETY PRECAUTIONS

- Swab sample should be considered biohazard material and treated as such on account of its potential pathogen content or health and life-threatening substances. While working with a swab sample it is essential to follow all safety requirements regarding work with biohazard material.
- It is recommended to carrying out the entire isolation procedure in the Class II Biological Safety Cabinet or at a laboratory burner as well as wearing disposable gloves and a suitable lab coat.
- It is recommended to use sterile RNase-free pipette filter tips.
- Avoid RNA transfer between minicolumns.
- Guanidine salts' residues may form highly reactive compounds when combined with oxidation components. In case of spillage, clean the surface with a detergent-water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

RNase elimination

RNases are very active enzymes which do not require any cofactors and are resistant to 15 minutes autoclaving at 121°C. In order to avoid the enzymes' degrading effect on RNA, it is essential to follow the recommendations below:

a. Use disposable gloves at all times when working with RNA. Do not come in contact with any items that are not specifically designed to work with RNA.
b. If possible, keep the samples at 2–8°C at all stages of the procedure, including centrifugation. Use decontaminated freezing racks instead of ice in order to avoid RNase contamination. Keeping RNA, after elution, in the freezing racks is recommended.
c. Plastic disposables (tips, tubes) should be RNase-free or autoclaved at 134°C for 18–20 minutes.
d. Reusable plastic, glass and porcelain should be soaked overnight in 0.1 N NaOH/0.1% DEPC water (or RNase-free water) and then washed with 0.1% DEPC water (or RNase-free water). When applicable, glass and porcelain (mortars) should be parched at 150–140°C for 2–4 h and cooled to room temperature.
e. Wipe surfaces, pipettes, centrifuge (rotor should be wiped separately) and tube racks with 3% hydrogen peroxide or <0.5% sodium hypochlorite (or any commercially available RNase inactivating fluid). Prior to decontamination, test the decontaminant on a small area of the material for possible undesired reactions.

Although the isolation using the ***EXTRACTME* VIRAL RNA KIT** guarantees high purity RNA without RNases contamination, using RIBOPROTECT RNase Inhibitor in furter applications might be necessary, to avoid RNA degradation during operations with RNA e.g. reverse transcription reaction set up.
The addition of RNase Inhibitor is possible directly after isolation, in proportion as follows: 0.5–1 Unit of RIBOPROTECT RNase Inhibitor (Cat. No. RT35) per 1 µl of the RNA eluate.

RNA elution

The optimal volume of the elution buffer vREB (viral RNA Elution Buffer) used should be chosen in accordance to the amount of the sample material and the final RNA concentration expected. The use of 30 µl vREB is recommended. The use of RNase-free water is optional.
The vREB buffer does not contain EDTA, which may interfere with some enzymatic reactions.

RNA storage and stability

For a long- term storage keep RNA at -80°C or in liquid nitrogen. The high quality and purity of eluted RNA allows to maintain its integrity during a short-term storage at -20°C.

DNA contamination

All biological material used for RNA isolation contains DNA. There is no RNA isolation method that may guarantee complete DNA removal unless the RNA sample is treated with DNase after isolation. Even slight DNA contamination (several gDNA copies per reaction) may give an additional signal in a quantitative PCR analysis after reverse transcription.

IX. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit. Do not mix **vRLys Buffer** vigorously.
2. Ensure that alcohol has been added to **vRW Buffer**. If not, add an appropriate amount of **isopropanol** (volumes can be found on the bottle labels or in the table given in section II).
3. Examine the **vRLys Buffer**. If a sediment occured in it, incubate it at 50°C mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. If possible, keep the samples at 2–8°C at all stages of the procedure, including centrifugation. Use decontaminated freezing racks instead of ice in order to avoid RNase contamination. Keeping RNA, after elution, in the freezing racks is recommended.

OPTIONAL:

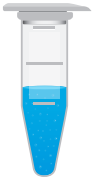
1. Prepare **freezing rack** to store the eluted RNA.

X. ISOLATION PROTOCOL

STEP 1

Transfer 100 µl of the transport medium containing a swab sample to a 1.5 ml Eppendorf tube. Add **400 µl vRLys Buffer** and vortex for 10 s.

STEP 2



Incubate the sample for 5 min at room temperature. Vortex several times during incubation.

STEP 3

Add **250 µl isopropanol** and mix by inverting several times.

STEP 4

Transfer the **obtain mixture** into a **RNA Purification Column** placed in a collection tube. Centrifuge for 30 s at 12 000 x g. Discard the filtrate and reuse the column together with the collection tube.

12 000 x g

30 s

STEP 5

Add **700 µl vRW Buffer** and centrifuge for 30 s at 12 000 x g.

Discard the filtrate and reuse the collection tube.

Repeat step 5.

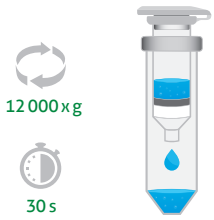
12 000 x g

30 s

12 000 x g

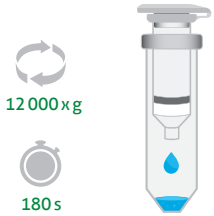
30 s

STEP 6



Add **300 µl vRW Buffer** and centrifuge for 30 s at 12 000 x g. Discard the filtrate and reuse the collection tube.

STEP 7



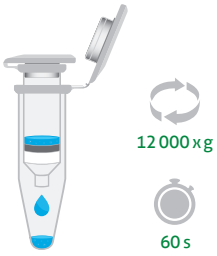
Centrifuge for 180 s at 12 000 x g (preferably at 15 000 x g). Discard the collection tube and the filtrate and carefully transfer the purification minicolumn to a sterile RNase-free 1.5 ml Eppendorf tube.

⚠ The vRW Buffer contains alcohol, which may interfere with some enzymatic reactions and decrease the elution efficiency. It is therefore crucial to remove the alcohol completely from the minicolumn before elution.

STEP 8

Add **30 µl** elution buffer **vREB**. Incubate minicolumn for 180 s at room temperature. Centrifuge for 60 s at 12 000 x g to elute purified RNA.

The isolated RNA is ready for use in downstream applications.



⚠ Keep isolated RNA at -20°C. For a long- term storage keep RNA at -80°C or in liquid nitrogen.

XI. TROUBLESHOOTING

Problem	Possible cause	Solution
RNA Purification Column becomes clogged during purification.	The purification column is overloaded.	Decrease the amount of a sample material.
Low RNA yield.	Sample material was incorrectly stored or preserved: RNA degradation.	Ensure that the swab was taken correctly and that the transport conditions were adequate.
	Too little sample material was used.	Take more sample material. A proper amount of a sample is dependent on the kind of a material examined and needs to be optimized individually.
	The purification column has become clogged.	See "RNA Purification Column becomes clogged during purification".
	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
	RNA is still bound to the column membrane.	Repeat the RNA elution.
Low purified RNA concentration.	Too much of elution buffer was used.	Decrease the vREB volume up to 30 µl.
Too low A_{260}/A_{230} ratio of purified RNA.	Remainings of buffers present in the eluate.	Ensure that the purification column had been properly dried before elution and no droplets remained on the ring. If necessary, increase centrifugation speed at step 7 of Isolation Protocol (section X) to 18 000 x g. Carefully remove the column from a collection tube.
	Incomplete sample loading.	Make sure that the lysate has passed completely through the RNA Purification Column before proceeding through washing steps. If necessary, increase centrifugation speed at step 4 of Isolation Protocol (section X).
Purified RNA is degraded.	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
DNA contamination present.	Too much sample material was used.	Decrease the amount of sample material. Optionally, the purified RNA sample may be treated with a DNase.

XII. SAFETY INFORMATION

vRLys Buffer

Danger
H302, H331, H412
P261, P271, P273, P304+P340 P311, EUH032

EUH032 Contact with acids liberates very toxic gas. **H302** Harmful if swallowed. **H331** Toxic if inhaled. **H412** Harmful to aquatic life with long lasting effects. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P271** Use only outdoors or in a well-ventilated area. **P273** Avoid release to the environment. **P304+P340 P311** IF INHALED: remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor.

XIII. RELATED PRODUCTS

Product	Blirt Cat. No.
RIBO*PROTECT* – RNase Inhibitor	RT35
Proteinase K	RP103
Reverse Transcriptase	RT32
TaqNovaHS DNA Polymerase	RP905A
DNaseMe – dsDNase	EN33