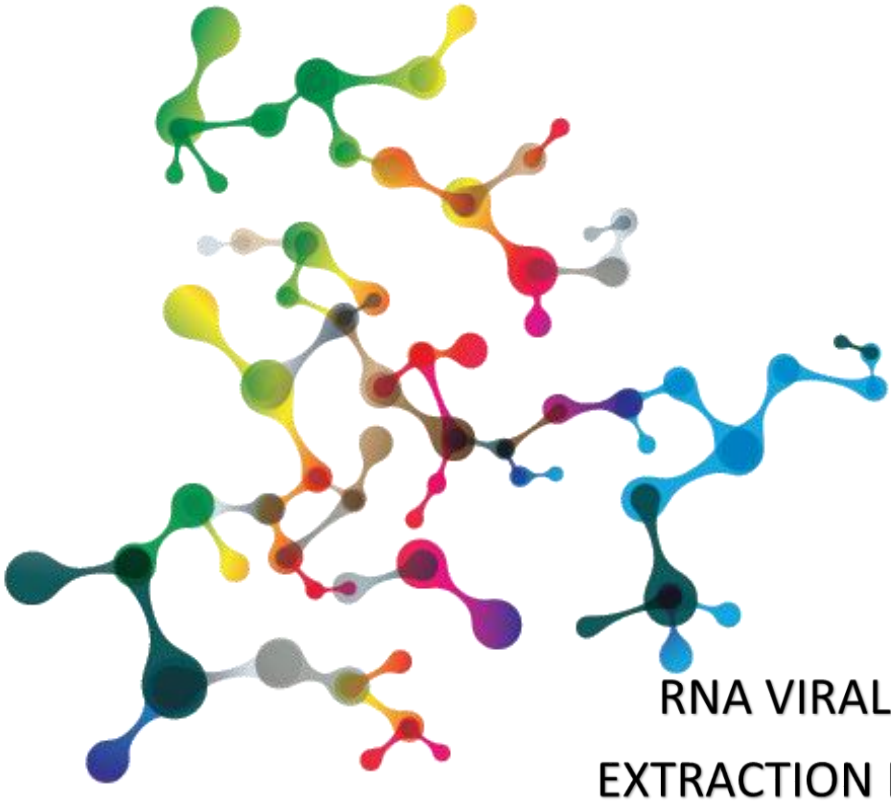
BIOPREMIER



RNA VIRAL EXTRACTION KIT

BIOPEXT-0613

CONTACT

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BPMR is certified ISO 9001:2015

INTRODUCTION AND PRODUCT DESCRIPTION

The Biopremier RNA VIRAL EXTRACTION Kit is designed for the rapid simultaneous purification of viral RNA from cell–free samples, solid samples, swabs and transport media. The viral lysis buffer contains detergents that lyse and inactivate RNases ensuring the extraction of intact viral RNA. Then, in the presence of a chaotropic salt, viral nucleic acids bound selectively to glass fiber membrane in a special centrifuge tube. The nucleic acids remain bound while a series of a rapid wash and spin steps removes contaminating cellular components. Finally, low salt elution removes the viral nucleic acids from the glass fiber membrane. The process does not require nucleic acids precipitation, organic solvent extractions, or extensive handling of the nucleic acids.

The Biopremier RNA VIRAL EXTRACTION Kit can be used for the isolation of viral RNA from a broad range of RNA virus. However, performance cannot be guaranteed for every virus species and must be validated by the costumer.

Applications:

- **Rapid isolation of high-quality, ready-to-use viral RNA.**
- **No organic extraction or alcohol precipitation.**
- **Complete removal of contaminants and inhibitors.**
- **The viral RNA can be used directly as templates for standard PCR or RT-PCR.**
- **Sample material: 200 µL serum, plasma, cell-free biological fluids.**

CONTENTS AND STORAGE

Name Tube	100 preps	Storage
Viral Lysis buffer	45 mL	RT
Protein Precipitation Buffer	4 mL	RT
Wash Buffer Virus 1	10 mL	RT
Wash Buffer Virus 2 *	20 mL	RT
Elution Buffer	8 mL	RT
Spin columns RNA	100 units	RT
Collection Tubes	200 units	RT

If properly stored, see the expiration date for the stability of the Kit. RT: room temperature.

Notes

(*)These solutions must be prepared as indicated in the section of preliminary preparations.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Microcentrifuge tube (1.5 – 2.0 mL)
- Micropipettes and micropipette filter tips (10 – 100 µL and 100 – 1000 µL)
- Vortex
- Microcentrifuge, able to operate up to 11.000 g.
- Powder-free gloves
- Ethanol 100 %

WARNINGS AND PRECAUTIONS

These products are exclusively for in vitro use

The test requires qualified staff to prevent the risk of erroneous results

Do not mix reagents from different batches

Do not use reagents from other manufacturer's products

Wear disposable gloves, laboratory coats when handling specimens and reagents.

Use sterile pipette tips with filters

Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately

Material Safety Data Sheets (MSDS) are available on request

Waste must be treated and disposed of in compliance with the appropriate safety standards

Clean periodically the working space with at least 5% of sodium hypochlorite or other ready-to-use solutions for eliminating RNase, DNase, and nucleic acid contamination.

It is strongly recommended to have dedicated areas, materials, and equipment for the RNA extraction, preparation of the PCR and post-PCR procedures

KIT USAGE INFORMATION

The kit contents should be mixed slightly before use.

Under cool environmental conditions, a precipitate may form in the Lysis buffer. In this case, the component should be heated to dissolve precipitate approximately 5 minutes at 37^{o}C and thoroughly shaken prior use.

PROCEDURE

PRELIMINARY PREPARATIONS

⚠ Viral Lysis Buffer and Wash Buffer Virus 1 contain guanidine isothiocyanate, which can form reactive components when combined with bleach. Both buffers are irritating agents, for this reason we recommend the use of gloves and glasses for handling. In case of contact with skin or eyes, wash with plenty of water

⚠ Add 80 mL of Ethanol 100 % to the Wash Buffer Virus 2. Keep the container closed to avoid the ethanol evaporation

SAMPLES

Plasma / serum: After obtaining the plasma or serum, it is important to centrifuge the sample to obtain a cell-free starting material.

Solid samples such as tissues/plants (5-10 mg): Homogenized in 300-400 µL of PBS using a hand-held electric homogenizer or ball-based homogenization systems. Centrifuge the sample and use 200 µL of the particle-free transparent supernatant.

Feces: Prepare a suspension with PBS, 10% (w/v) and use the best system to be able to lyse all the viral particles. Centrifuge the sample and use 200 µL of the particle-free transparent supernatant.

Swabs: Incubate the swab in an adequate amount of buffer (ex. PBS) for 30 minutes with movement. Remove the swab and proceed with 200 µL of the particle free transparent supernatant.

Transport Medium/Viral Transport Medium: Vortex the tubes containing the swab at maximum speed for 1 minute. Use 200 µL as the input sample.

NOTE: In tissues and feces it should be considered that other RNAs can be copurified that can inhibit the following PCR assays.

PROTOCOL FOR THE EXTRACTION OF VIRAL RNA

1. **Add 200 µL of sample** into a microtube. If you process samples less than 200 µL, adjust the final volume to 200 µL using PBS or nuclease-free water.

2. **Add 400 µL Viral Lysis Buffer**. Close the microtube and vortex vigorously for 20 seconds.

3. **Incubate at room temperature for 10-15 minutes.**

NOTE: If the sample is very viscous (sputum), the use of proteinase K or incubation at 70ºC is recommended.

NOTE: The incubation time and temperature is critical for lysis as well as for RNA stability. Incubation at room temperature is usually sufficient without significant loss of sensitivity. Depending on the kind of sample, an optimization of the previous points will be recommended (compare the addition of proteinase K, incubation times and temperatures).

4. Add **30 µL of Protein Precipitation Buffer**. Vortex and incubate for 1 minute.

NOTE: If you are purifying virus transport medium samples, you can improve the yield by not adding the Protein Precipitation Buffer. Skip step 4 and proceed directly with centrifugation on the step 5.

5. **Centrifuge at 11.000 g for 3 minutes.**

6. **Transfer the supernatant to** a new microtube avoiding touching the pellet that can form.

7. Add **350 µL Ethanol 100%**. Mix well.

8. Place the spin column in a 2 mL collection tube.

9. Transfer **600 µL** of the mix to the column. **Centrifuge at 8.000 g for 30 seconds.**

10. Repeat step 9 to **load the remaining sample**.

11. Place the spin column in a new collection tube.

12. Add **100 µL of RNA Wash Buffer Virus 1**. Centrifuge at 11.000 g for 1 minute.

13. Add **700 µL of RNA Wash Buffer Virus 2**. Centrifuge at 11.000 g for 1 minute.

14. Centrifuge **for 3 minutes at 11.000 g** to completely dry the membrane.

15. Remove the collection tube and insert the Spin column in a clean 1.5 mL microtube.

16. Add carefully to the centre of the column membrane **50 µL of Elution Buffer or nuclease-free water**. Incubate at room temperature for 2 minutes.

17. Centrifuge at **11.000 g for 1 minute**. Collect the 50 µL and re-deposit in the center of the membrane. This increases the performance.

18. Incubate at room temperature for 2 minutes and centrifuge at **11.000 g for 1 minute**.

19. Store viral RNA at -80 °C or use it in applications immediately.

WORKFLOW – SAMPLE PREPARATIONS

1 – Plasma / serum

		PROCEDURE Plasma / serum
1 SAMPLE PREPARATION		>10.000 x g - Collect 200 µL - cell-free starting material

2 – Solid samples

		PROCEDURE Solid samples
1 SAMPLE PREPARATION		Weight (5-10 mg) and homogenize in 300-400 µL of PBS >10.000 x g - Collect 200 µL

3 – Feces

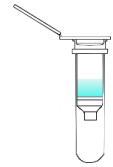
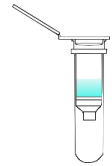
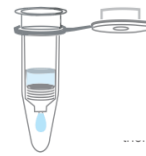
		PROCEDURE Feces
1 SAMPLE PREPARATION		Prepare suspension with PBS, 10% (w/v) and homogenize >10.000 x g - Collect 200 µL- cell-free starting material

4 – Swabs

	PROCEDURE Swabs
1 SAMPLE PREPARATION 	Incubate in a buffer (ex. PBS), 30 min. shaking Collect 200 µL

5 – Transport Medium / Viral Transport Medium

	PROCEDURE Transport Medium / Viral Transport Medium
1 SAMPLE PREPARATION 	Vortex, max speed, 1 min. Collect 200 µL

		PROCEDURE
1 SAMPLE PREPARATION		Collect 200 µL of sample Check in detail the **WORKFLOW – SAMPLE PREPARATIONS** procedures for each type of sample.
2 LYSE CELLS		400 µL Lysis buffer Vortex RT (25ºC), 10-15 min Optional: add proteinase K / incubation at 70ºC
3 PRECIPITATION BUFFER		30 µL of Precipitation Protein Buffer Vortex Incubate RT (25ºC), 1 min 11.000 x g, 3 min Take of supernatant Optional: skip this step for virus transport medium samples
4 BIND		Supernatant (step before) 350 µL of Ethanol 100% Mix Load the spin column (max. 600 µL), 8.000 x g, 30 sec (repeat)
4 WASH		100 µL of RNA Wash Buffer 1 11.000 x g, 1 min 700 µL of RNA Wash Buffer 2 11.000 x g, 1 min 11.000 x g, 3 min
5 ELUTE RNA		50 µL of Elution Buffer Incubate RT (25ºC), 1 min 10.000 x g, 1 min Collect 50 µL and re-deposit. Incubate RT (25ºC), 1 min 10.000 x g, 1 min

TROUBLESHOOTING

Trouble	Possible Reason	Solution Suggest
Low RNA yield or low RNA purity	Inappropriate storage conditions	The Kit should be stored between +15 and +25^{o}C. The tube and bottle caps must be tightly sealed after each use to maintain the pH values and stability of the kit components, and to prevent contamination
	Chemicals and sample are not mixed well	The sample should be thoroughly mixed after each chemical addition
	Poor elution	Repeat the elution step. Collect the eluted RNA and and re-deposit in the center of the membrane to ensure better RNA capture
	No glove switching or use	Since this is a kit to extract RNA, glove changing is important to reduce RNase activity and loss of yield
	Starting cells or tissue contains small amounts of RNA	The yields may vary greatly depending different types of tissues
	Tissue or culture is too old	Use cultures before they reach maximum density or become fully confluent and harvest tissues as rapidly as possible
	Tissue may be RNAse- rich	Some tissues can be RNAse- rich and may require immediate action and disruption by the Viral Lysis Buffer to prevent degradation
	Insuficient disruption	Vortex or pipette lysate until no cell clumps remain. Homogenize tissues in the Viral Lysis Buffer until no visible particles remain
	Improper storage	The samples must be kept at very low temperatures to inhibit RNAse activity
No amplification after RTqPCR run	Alcohol residue in RNA isolate	The remaining ethanol after washing steps should be removed by centrifuging the column at >10.000 g for 3 minutes
Unacceptable level of DNA contamination in purified RNA	Binding column overloaded	Use fewer cells or smaller tissue samples

QUALITY CONTROL

Each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

TRADEMARK, DISCLAIMER AND PRODUCT USE RESTRICTION

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

The use of this product signifies the agreement of any purchaser or user to the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

The kit components are intended exclusively for *in vitro* use, and for research purposes only! BPMR products are intended for general laboratory use only! Molecular Biology procedures, such as RNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results. It is strongly recommended to have dedicated areas, materials and equipment for the RNA extraction, preparation of the PCR and post-PCR procedures. The workflow in the laboratory should proceed in a uni-directional manner, from the Extraction Area to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed the previous step. The user should always read all the instructions provided with the product before running the assay. Not mix reagents from different batches. Not use reagents from other manufacturer's products. Wear disposable gloves, laboratory coats when handling specimens and reagents. Use sterile pipette tips with filters. Waste must be treated and disposed of in compliance with the appropriate safety standards.

ADDITIONAL INFORMATION

For additional information, technical support or troubleshooting please contact: tech.support@biopremier.com

ORDERING INFORMATION

Biopremier offers a large selection of products. Visit www.biopremier.com or contact sales.support@biopremier.com for more detailed product information.

Reference	Product	Quantity
BIOPSFS-sp0064	SUPREME RT-PCR Detetion Kit HLVd	100 rxn
BIOPSFS-0064	SUPREME RT-PCR Detetion Kit HLVd	500 rxn
BIOPSFS-sp0065	SUPREME RT-PCR Detetion Kit HLVd/LCV/CanCV	100 rxn
BIOPSFS-0065	SUPREME RT-PCR Detetion Kit HLVd/LCV/CanCV	500 rxn