RNA Isolation Kit Plasma/Serum

50 Preps

Product Data Sheet

Cat. No.: RIK002

For Research Use Only

BioVendor
Research
and Diagnostic Products

CONTENTS

This kit is manufactured by:

BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The RIK002 RNA Isolation Kit Plasma/Serum is an isolation kit designed for the purification of small and large RNA including miRNA from cell free blood plasma and serum.

FEATURES

- It is intended for research use only
- Purification of total RNA including miRNA
- Optional separation of small RNA (<200 nt) and large RNA (>200 nt) fractions
- Excellent RNA recovery and purity
- Fast isolation – approximately 45 minutes
- No phenol/chloroform extraction necessary
- RNA product suitable for sensitive downstream applications

SPECIFICATIONS

- Silica-membrane technology
- Mini spin columns
- Fragment size: ≥ 18 nt
- Binding capacity 200 µg
- Elution volume 20–50 µl

2. STORAGE AND EXPIRATION

Store the complete kit at room temperature (RT). Under these conditions, all components are stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

The RNA Isolation Kit Plasma/Serum kit offers the unique feature to isolate total RNA including small RNA and DNA from serum and plasma without the need to resort to the cumbersome phenol / chloroform extraction or a time consuming proteinase digest.
The sample material is denatured in Lysis Buffer LBP. The protein is then precipitated by Protein Precipitation Buffer PPBP and pelleted by centrifugation.
After the removal of protein the binding conditions for nucleic acids are adjusted by adding isopropanol.
Total nucleic acids are bound to the isolation column. Optionally, DNA can be removed by an on-column rDNase digest. The remaining nucleic acids are washed and eluted with minimal amounts of RNase-free water.
The standard procedure allows to process 300 µl of sample material with only one loading step onto the isolation column. This is usually enough to detect also low abundance miRNA in plasma or serum.
If larger sample volumes are to be used to increase the sensitivity even further, the volumes of Buffer LBP and Buffer PPBP as well as the isopropanol have to be increased proportionally. Multiple loading steps per sample are necessary.

4. PRECAUTIONS

- **For professional use only**
- This kit contains hazardous contents
- Avoid contact with the rDNase, RNase-free, LBP and WB1P, which may cause skin/eyes irritation and harmful if swallowed. In case of contact with the rDNase, RNase-free, LBP and WB1P, wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

5. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- **RNases are stable and robust enzymes that catalyze degradation of RNA. It is therefore indispensable to create an RNase-free environment following the rules summarized below:**
  - The RNase-free working environment should be located away from microbiological work station
  - Use disposable gloves when handling reagents, samples, pipettes, and tubes
  - The gloves should be changed frequently to avoid contamination
  - Tips, tubes, lab coats, pipettes, etc. should be allocated for RNA work only
  - Nuclease-free water should be used
  - Commercial RNase decontamination solution should be used to clean all surfaces
  - Isolated RNA samples should be kept on ice
  - Use filtered pipette tips
  - Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- Avoid any contamination among samples and reagents. For this purpose, disposable tips must be used for each sample and reagent.
- Dispose consumable materials and unused contents in accordance with applicable national regulatory requirements

6. REAGENTS SUPPLIED

Kit Components	*State*	*Quantity*
Lysis Buffer (LBP)	ready to use	13 ml
Protein Precipitation Buffer (PPBP)	ready to use	5 ml
Reaction Buffer for rDNase	ready to use	7 ml
rDNase, RNase-free	lyophilized	1 vial
Wash Buffer (WB1P)	ready to use	10 ml
Wash Buffer Conc. (WB2P)	concentrated	25 ml
RNase-free H_2O	ready to use	13 ml
Isolation Columns	ready to use	50 pc
Collection Tubes (1.5 ml)	ready to use	50 pc
Collection Tubes (2 ml)	ready to use	50 pc
Collection Tubes (2 ml, lid)	ready to use	50 pc
Product Data Sheet + Certificate	-	1 pc

7. MATERIAL REQUIRED BUT NOT SUPPLIED

Reagents

- Ethanol 96–100 %
- Isopropanol
- Proteinase K
- Syntetic Carrier

Equipment/Consumables

- Commercially available RNase decontamination solution
- Disposable gloves, goggles
- Test tubes for diluting samples (nuclease-free PCR tubes)
- Nuclease-free, low nucleic acid binding tubes (1.5 ml)
- Glassware (graduated cylinder and bottle) for Wash Solution and RNase Inhibitor Solution
- Precision pipettes to deliver 5–1000 µl with disposable filter pipette tips (nuclease-free)
- Vortex mixer
- Centrifuge

8. PRE-ANALYTICAL PHASE

Sample Type

RNA Isolation Kit Plasma/Serum is validated for isolation from cell free blood plasma and serum.

Processing of plasma from human EDTA blood

Conditions during sample collection may affect the isolation process. Therefore, it is highly recommended to follow standardized procedure for blood collection:

- Standardized needles and EDTA blood collection tubes are needed
- Gloves must be worn all the time when handling specimens
- Centrifuge fresh blood sample for 10 min at 2,000 x *g*

- Remove the plasma without disturbing sedimented cells
- Freeze plasma at -20 ℃ for storage upon RNA isola tion
- Thaw frozen plasma samples prior to RNA isolation and centrifuge for 3 min at ≥ 11,000 x *g* in order to remove residual cells, cell debris, and particulate matter
- Use the supernatant for RNA isolation

Processing of serum

The selection of a collection tube is left to an individual's discretion as long as it is without additives and designated for serum isolation by the manufacturer. Serum samples that are allowed to sit less than 30 min are likely to retain cellular elements and other contaminants impacting future analysis. Samples that sit longer than 60 min are likely to experience lysis of cells in the clot, releasing cellular components not usually found in serum samples.
Recommended standardized procedure:

- Centrifuge fresh blood sample for 10 min at 2,000 x *g*
- Remove the serum without disturbing sedimented cells
- Freeze serum at -20 ℃ for storage upon RNA isolat ion
- Thaw frozen serum samples prior to RNA isolation and centrifuge for 3 min at ≥ 11,000 x *g* in order to remove residual cells, cell debris, and particulate matter
- Use the supernatant for RNA isolation

Stability and Storage:

- Avoid repeated freeze-thaw cycles
- Samples should be stored at -20°, or preferably at -70℃ for long-term storage.

9. PREPARATION OF REAGENTS

- **All reagents need to be brought to room temperature prior to use**
- **Always prepare only the appropriate quantity of reagents for your test**
- **Do not use components after the expiration date marked on their label**
- **Wear gloves and goggles**

ASSAY REAGENTS SUPPLIED READY TO USE

Lysis Buffer (LBP)
Protein Precipitation Buffer (PPBP)
Reaction Buffer for rDNase
Wash Buffer (WB1P)

Stability and storage:
Opened reagents are stable for at least 12 months when stored at room temperature (18–25℃). Storage at lower temperatures may cause precipitation of salts. If precipitation occurs, incubate the bottle for several minutes at about 30–40℃ and mix well until the precipitate is redissolved.

ASSAY REAGENTS SUPPLIED CONCENTRATED OR DRIED

rDNase, RNase free (lyophilized)

Reconstitute the lyophilized rDNase, RNase free with 3 ml Reaction Buffer for rDNase and incubate for 1 min at room temperature. Let it dissolve and gently shaking the vial (not to foam). Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 ℃.

Stability and storage:
The frozen solution is stable for at least 6 months. Do not freeze / thaw the aliquots more than three times.

Wash Buffer Conc. (WB2P)

Add 100 ml of 96–100% ethanol to the 25 ml WB2P Concentrate and mix well.

Stability and storage:
The buffer can be stored at room temperature (18-25℃) for at least one year.

10. PURIFICATION PROTOCOL

Before starting with the preparation check that isopropanol is available, that ethanol was added to Wash Buffer WB2P, and that rDNase was reconstituted according to Chapter 9.

1 Preparation of sample: Add **90 µl Buffer LBP** to **300 µl sample**.
Vortex for **5 s**.
Incubate for **3 min** at **room temperature (18-25°C)** .

Note: To process 600 µl or 900 µl sample material, increase volumes for Buffer LBP, PPBP, and isopropanol proportionally. Multiple loading steps will be necessary in step 5.

2 Precipitate protein: Add **30 µl Buffer PPBP** and vortex for **5 s**.
Incubate for **1 min** at **room temperature (18-25°C)** .
Centrifuge for **3 min** at **11,000 x *g*** to pellet the protein.

3 Transfer supernatant: Transfer the clear supernatant into a new Collection Tube (2 ml, lid).

4 Adjust binding cond.: Add **400 µl isopropanol** and vortex for **5 s**.

Note: Addition of carrier, for example, 2 µg of glycogen or 5 µg of LPA (linear polyacrylamide), might slightly improve the miRNA yield but usually is not necessary. Poly-A has shown only negligible effects and furthermore might interfere with photometric miRNA quantification.

5 Bind RNA and DNA: Place an **isolation column** in a Collection Tube (2 ml) and load the sample onto the column.
Incubate for **2 min** at **room temperature (18-25°C)** .
Centrifuge for **30 s** at **11,000 x *g***.
Discard the flow-through and place the column back into the collection tube.

Note: If more than 300 µl plasma / serum was used, repeat this step until all sample is loaded onto the column.

6 Optional: Wash: Add **700 µl Buffer WB2P** to the isolation column
Centrifuge for **30 s** at **11,000 x g**. Discard flow through and place the column back into the collection tube.

Add **250 µl Buffer WB2P** to the isolation column
Centrifuge for **2 min** at **11,000 x *g***. It is not necessary to discard the flow-through.

Note: Co-purified DNA might interfere with qPCR quantification of miRNA. The following on-column digest degrades bound DNA including miRNA genes.
However, if miRNA specific qPCR detection systems are used or cell free plasma DNA is needed intact for further analysis, skip the rDNase digest and proceed directly with step 7.

Digest DNA: Add **50 µl rDNase** (dissolved in Reaction Buffer for
rDNase according to section 3) directly onto the silica membrane of the isolation column.
Close the lid and incubate at **room temperature (18-25°C)** for **15 min**.

7 Wash and dry silica membrane:

Add **100 µl Buffer WB1P** to the isolation column.
Centrifuge for **30 s** at **11,000 x *g***.
Discard flow-through and place the column back into the collection tube.
Add **700 µl Buffer WB2P** to the isolation column.
Centrifuge for 30 s at **11,000 x *g***.
Discard flow-through and place the column back into the collection tube.
Add **250 µl Buffer WB2P** to the isolation column.
Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane completely.
If the liquid in the collection tube has touched the isolation column after the 3rd wash, discard flow-through and centrifuge again.

Note: The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If new collection tubes are to be used for each step, see ordering information.

8 Elute RNA and DNA: Place the isolation column in a new Collection Tube (1.5 ml).

Note: The elution buffer volume highly influences the final yield and concentration and, furthermore, influences elution efficiency of large oligonucleotides. See Chapter 11 for more information about elution in 20, 30 or 50 µl.

Add **30 µl RNase-free H_2O** directly onto the silica membrane of the column.

Incubate for **1 min** at **room temperature (18-25℃)** .
Close the lid and centrifuge for **1 min** at **11,000 x *g***.

9 Use eluted RNA and DNA:

Use fresh isolate samples immediately in downstream applications or freeze isolate samples for later use.

Note: The total concentration of RNA can be determined by measuring the absorbance at 260 nm in a spectrophotometer.

Stability and storage of isolates:

Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20℃, for long term storage freeze at -70℃. RNA samples shou ld be stored in nuclease-free plastic tubes. Avoid freeze-thaw cycles of isolated samples.

Elution Procedure

The elution buffer volume does not only influence total yield and concentration of RNA and DNA, but does also influence the ratio between very small and larger oligonucleotides:

20 µl
The silica membrane is not completely wetted. Only weakly binding very small oligonucleotides like miRNA are eluted efficiently. Larger RNA and DNA are more likely to remain bound to the column. The eluted miRNA is highly concentrated.

30 µl (standard)
The standard elution buffer volume of 30 µl is sufficient to wet the silica membrane completely. It results a high total yield of miRNA/RNA/DNA and simultaneously maximizes the concentration.

50 µl
Increasing the elution buffer volume will further increase the final yield but consequently will reduce the concentration. The gain in yield will usually not compensate for the loss in sensitivity of miRNA detection caused by the dilution of the eluate. Furthermore, larger RNA and DNA will be eluted more efficiently, which might, however, be interesting for the analysis of circulating DNA.

Proteinase K digest

A short protein digestion step might increase miRNA yield, especially for low quality, hemolyzed plasma. Add a sufficient quantity of Proteinase K to the sample (plasma/serum).

Normalization

For monitoring the efficiency of isolation, it is recommended to add a defined amount of synthetic nonhuman RNA.

Poor or no RNA yield

Addition of carrier
• To improve RNA/DNA binding to the isolation column, carrier can be added to the sample after the removal of precipitated protein. Slightly higher yields could be found with 2 µg of glycogen or 5 µg of LPA (linear polyacrylamide).

However, negligible effects were observed for Poly-A which furthermore interferes with a photometric quantification of the purified nucleic acids.

Reagents not applied or restored properly

• Always dispense exactly the buffer volumes given in the protocols! The correct proportions of buffers LBP, PPBP, and isopropanol are essential for optimal yield and purity.

• Always follow closely the given instructions with regard to order and mode of mixing (shaking, vortexing etc).

• Add the indicated volume of 96–100% ethanol to Buffer WB2P Concentrate and mix thoroughly.

• Store kit components at room temperature (18–25℃). Storage at lower temperatures may cause salt precipitation. Heat buffer with precipitated salt to 30℃ until salt is dissolved. Let the buffer cool down to room temperature before use.

• Keep bottles tightly closed in order to prevent evaporation or contamination.

RNA is degraded

RNase contamination

• Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use sterile, disposable polypropylene tubes and filter tips. Keep tubes closed whenever possible during the preparation unless stated otherwise. Glassware should be oven-baked for at least 2 hours at 250 ℃ before use.

Suboptimal performance of RNA in downstream experiments

Inhibition by co-purified RT-PCR inhibitors

• Heme, hemin, and other degradation products of red blood cells strongly inhibit reverse transcription and PCR. Too much plasma or bad plasma quality can result in contamination with these inhibitors. Use less plasma, dilute eluates, perform the
optional Proteinase K digest, or add BSA prior to RT or PCR reactions.

Carry-over of ethanol or salt

• Do not let the flow-through touch the column outlet after the second WB2P wash. Make sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic buffer WB2P completely.

• Check if buffer WB2P has been equilibrated to room temperature (18-25℃) before use. Washing at lower temperatures lowers efficiency of salt removal.

12. EXPLANATION OF THE SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	Attention, see instructions for use
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Purification Protocol – summary

Prepare **samples:**	300 µl plasma or serum 90 µl LBP Vortex 5 s RT, 3 min	
Precipitate **protein:**	30 µl PPBP Vortex 5 s RT, 1 min 11,000 x *g*, 3 min	
Transfer **supernatant:**	Transfer clear supernatant to Collection Tube (2 ml, lid)	
Adjust **binding cond.:**	400 µl isopropanol Vortex 5 s	
Bind **RNA and DNA:**	Load sample on isolation column RT, 2 min 11,000 x *g*, 30 s	
Optional: **Digest DNA**	700 µl WB2P 11,000 x *g*, 30 s 250 µl WB2P 11,000 x *g*, 2 min 50 µl rDNase in Reaction Buffer for rDNase RT, 15 min	
Wash and dry:	100 µl WB1P 11,000 x *g*, 30 s 700 µl WB2P 11,000 x *g*, 30 s 250 µl WB2P 11,000 x *g*, 2 min	
Elute RNA:	30 µl RNase-free H2O RT, 1 min 11,000 x *g*, 1 min	

13. NOTES

There are BioVendor branches and distributors near you.
To find the office closest to you, visit **www.biovendor.com/contact**

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