RNA Isolation Kit

50 Preps

Product Data Sheet

Cat. No.: RIK001

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 RIK001 RNA Isolation Kit is isolation kit designed for the purification of small and large RNA including miRNA from cells, tissues and a general RNA reaction clean up. Optionally, protein and DNA can be isolated as well.

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 30–100 µl
- Sample requirements:
  < 10^7 cultured cells
  < 30 mg human/animal tissue
  < 50 mg plant tissue
  < 150 µl reaction mix

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 kit offers the unique feature to isolate total RNA including small RNA and DNA from cells, tissues and a general RNA clean up without the need to resort to the cumbersome phenol/chloroform extraction or a time consuming proteinase digest.
Sample material is lysed and stabilized in Lysis Buffer LB, containing denaturing salts and β-mercaptoethanol (step 1). Depending on the sample material, additional
mechanical disruption might be necessary to assist the disruption of hard-to-lyse cell
walls and thus to reduce the time intrinsic RNases are active. For lipid-rich or hard-to-lyse tissue a phenol-based lysing substance.
A filtration step with an inert filter removes unlysed remains of the sample and reduces the viscosity of the lysate (step 2). High molecular weight DNA is sheared and hereby prepared for a more efficient DNase digest. The filtrate is further processed while the filter column is discarded.
Addition of ethanol (step 3) adjusts binding conditions for the binding of large RNA and DNA fragments above approximately 200 nt to the isolation column (step 4) while small RNA of less than about 200 nt and proteins are in the flow-through. This separation of small and large nucleic acids is necessary to guarantee a superior RNA purity as DNA and proteins can each be removed separately in a patented and most efficient way. Both the isolation column with long nucleic acids and the flow-through containing short nucleic acids and proteins are kept at this step.
If purification of total nucleic acids including DNA is desired, the following steps 5 and 6 are skipped. Otherwise a desalting step with Buffer MDB (step 5) prepares the isolation RNA Column for the following on column DNA digest (step 6). During the ongoing DNase incubation, the flow-through of step 4, containing small RNA and protein, is further processed. Addition of Buffer PPB precipitates protein from the sample. Precipitated protein is removed by centrifugation (step 7). If desired, the protein pellet can be redissolved and analyzed. Remaining protein is removed from the supernatant via filtration through a isolation Protein Removal Column (step 8) leaving only small RNA in the flow-through. The isolation Protein Removal Column is discarded and the clear flow-through combined with Buffer BB which adjusts binding conditions for small RNA to the isolation RNA Column (step 9).
After the DNase digest is completed the mixture of step 9 is loaded stepwise into the
isolation RNA Column (step 10). If preparation of small and large RNA in separate fractions is desired, the mixture of step 9 can also be bound to a second isolation RNA Column instead. In this case the following washing steps are performed for both columns in parallel.

Stringent washing steps with Wash Buffers WB1 and WB2 remove DNA fragments,
contaminants and salts (step 11). An optional third washing step with Buffer WB2 removes trace amounts of chaotropic salt carryover. Ethanol from Wash Buffer WB2 is removed by a prolonged centrifugation step.
Pure RNA is eluted in 30–100 µl of supplied RNase-free H_2O at step 12. See Chapter 11 for details concerning the choice of elution volume. Eluted RNA is ready for standard downstream applications.

4. PRECAUTIONS

- **For professional use only**
- This kit contains hazardous contents
- Avoid contact with the rDNase, RNase-free, LB, BB, WB1 and MDB, which may cause skin/eyes irritation and harmful if swallowed. In case of contact with the rDNase, RNase-free, LB, WB1 and MDB, 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 (LB)	ready to use	30 ml
Protein Precipitation Buffer (PPB)	ready to use	20 ml
Binding Buffer (BB)	ready to use	60 ml
Membrane Desalting Buffer (MDB)	ready to use	25 ml
Reaction Buffer for rDNase	ready to use	7 ml
rDNase, RNase-free	lyophilized	2 vial
Wash Buffer (WB1)	ready to use	35 ml
Wash Buffer (WB2)	concentrated	12 ml
RNase-free H_2O	ready to use	13 ml
Isolation Filters	ready to use	50 pc
Isolation RNA Columns	ready to use	50 pc
Isolation Protein Removal 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	150 pc
Product Data Sheet + Certificate	-	1 pc

7. MATERIAL REQUIRED BUT NOT SUPPLIED

Reagents

- Ethanol 96–100 %
- Syntetic Carrier
- Optional: phenol-based lysis reagent

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
- Equipment for sample disruption and homogenization

8. PRE-ANALYTICAL PHASE

Sample Type

RNA Isolation Kit is validated for isolation from cells, human/animal tissue and plant tissue.

Amount of starting material

Ideally, the amount of starting material should be at the upper limit of the range in specifications (see Chapter 1) in order to achieve efficient purification of small and large RNA.
For quantitative RNA purification from starting material less than 3 mg tissue or 10^6 cells, it is advantageous to add 10 µg of Carrier RNA before binding of small RNA to improve RNA binding.

Preparation and storage of starting materials

RNA is not protected against digestion until the sample is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. For long term storage it is recommended that samples are flash frozen and stored at -70℃ to -80℃ as soon as possible.
After disruption, samples can be stored in Lysis Buffer LB at -70℃ to -80℃ for up to one year; at -20℃ for up to 6 months; at 2-8℃ for up to 24 hours; or up to several hours at room temperature. Frozen samples in Lysis Buffer LB should be thawed slowly until no remaining salt crystals are visible before starting with the RNA
isolation.
Wear gloves at all times during the preparation! Change gloves frequently! Use RNase-free equipment only!

Cultured tissue and cells

Cultured tissue and cells can be collected by centrifugation (after trypsinization, if necessary). The cell pellet can be redissolved in Lysis Buffer LB where cells are lysed almost immediately. Nevertheless, a change in expression profiles during long washing and centrifugation steps as well as during trypsinization must be considered. Optionally, adherent cells can be lysed directly in the culture flask. Remove culture medium and wash the cells with Phosphate Buffered Saline (PBS) before addition Lysis Buffer LB.

Cells grown in monolayer

Remove culture medium completely and wash cells once with Phosphate Buffered Saline (PBS). Lyse cells by addition of **300 µl Buffer LB** for each **$5x10^6$ cells** directly to the culture disk and incubate for **5 min** at **room temperature** (18-25 ℃).
or
Collect up to **10^7 cultured cells** after trypsinization by centrifugation, discard supernatant and add **300 µl Buffer LB**. Pipette up and down or vortex to lyse the cells.

Cells grown in suspension

Collect up to **10^7 cultured cells** by centrifugation, discard supernatant and add **300 µl Buffer LB**. Pipette up and down or vortex to lyse the cells.
In all cases transfer exactly **300 µl lysate** to an **Isolation Filters** (violet ring) in a Collection Tube (2 ml, lid).

Animal and plant tissue

Animal and plant tissue is often solid and might be protected by a cell wall, which reduces the effectiveness of lysis buffers. Therefore, mechanical assistance is essential to quickly break up the cells and stabilize the RNA in Buffer LB.

Bacteria and yeast

Bacteria and yeast have to be incubated in lysozyme or lyticase/zymolase solutions, respectively to break down the robust cell walls of these organisms. Sonication and mechanical disruption are alternatives for cell disruption. Avoid long incubation times to prevent changes in expression profiles.

Purification of total nucleic acids including DNA

The RNA Isolation kit is suitable for purification of **total nucleic acids including DNA** and large RNA/small RNA as well as for the purification of denatured protein. To enable a purification of DNA, the membrane desalting step with Buffer MDB and the DNase digest are omitted (steps 5 and 6). It is important **not** to perform the membrane desalting step with **Buffer MDB** if purification of DNA is desired.
A separation of total nucleic acids into DNA and RNA can be achieved by an enzymatic digest of the split and eventually aliquoted eluate with DNase and RNase.

Analysis of the protein fraction

Buffer ML contains high amounts of chaotropic salt and β-mercaptoethanol. Furthermore, ethanol is added prior to the protein precipitation resulting in completely denatured protein. The precipitated protein pellet of the recommended amount of starting material might be difficult to resuspend, so if protein analysis is required, it could be advantageous not to use the protein pellet of the complete sample for further analysis but to remove an aliquot of 10–20 µl lysate before addition Buffer PPB (between steps 4 and 6) and to precipitate this smaller portion with an adapted volume of Buffer PPB separately (6.7–13.3 µl Buffer PPB respectively).
Add 500 µl of 50% ethanol to the protein pellet (no resuspension necessary) and centrifuge for 1 min at 11,000 x *g*. Remove the ethanol completely and let the protein pellet dry at room temperature for 10 min.
Usually the denatured protein is dissolved in Laemmli buffer or a similar SDS-containing solution by incubating the sample at 90°C for at least 5 minutes.

Undissolved protein is removed by centrifugation and the solubilized protein can be used for downstream analysis.
Most protein quantification assays such as Bradford, Lowry, BCA, etc. Do not work in the presence of SDS.

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 – buffers LB, MDB, and WB1 contain chaotropic salt**
- **CAUTION: Buffer LB, MDB, and WB1 contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample–preparation waste!**

ASSAY REAGENTS SUPPLIED READY TO USE

Lysis Buffer (LB)
Protein Precipitation Buffer (PPB)
Binding Buffer (BB)
Membrane Desalting Buffer (MDB)
Reaction Buffer for rDNase
Wash Buffer (WB1)

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 each lyophilized rDNase, RNase free vial 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. (WB2)

Add 48 ml of 96–100% ethanol to the 12 ml WB2 concentrate and mix well.

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

10. PURIFICATION PROTOCOLS

Before starting with the preparation check that 96–100% ethanol is available, that ethanol was added to Wash Buffer WB2, and that rDNase was reconstituted according to Chapter 9.

Purification protocol 1:

RNA purification from animal tissue, plant material and cultured cells

1 Cell lysis:

Tissue and plant material

Thoroughly disrupt up to 30 mg of sample material in 300 µl Buffer LB using mechanical devices. If necessary, increase sample amount and lysis buffer volume proportionally. Optimal lysis conditions need to be evaluated for each sample material individually.

Cultured cells

Lyse up to 10^7 cultured cells in 300 µl Buffer LB.
Pipette up and down or vortex to lyse the cells.
Incubate 5 min at room temperature (18-25℃).

2 Homogenization of the lysate:

Place an Isolation Filters (violet ring) into a Collection Tube (2 ml, lid). Load the lysate and centrifuge for 1 min at 11,000 x g to reduce viscosity and to clear the lysate from undissolved debris.
If a pellet is visible in the Collection Tube (2 ml, lid) after the centrifugation, transfer the supernatant to a fresh centrifuge tube (not supplied) without disturbing the pellet.

Alternative: samples without debris can be homogenized by passing them through a 0.9 mm needle (20 gauge), fitted to a syringe.

Discard the Isolation Filters (violet ring) and proceed with the flow-through.

3 Adjust binding conditions for large RNA and DNA:

Add exactly 150 µl 96–100% ethanol to 300 µl flow–through from step 2.
Vortex immediately for 5 s.

Note: After addition of ethanol a precipitate may become visible. Do not remove the precipitate and load it into the column at step 4!

Incubate for 5 min at room temp. (18-25℃).

4 Bind large RNA and DNA:

Combine a Isolation RNA Column (blue ring) with a Collection Tube (2 ml, lid) and load the sample including any precipitate into the column.
Centrifuge for 1 min at 11,000 x g.
Keep both the Isolation RNA Column with bound large RNA and DNA and the flow–through containing small RNA and proteins!
Place the Isolation RNA Column in a new Collection Tube (2 ml) without lid. Close the lid of the Collection Tube (2 ml, lid) with the saved flow-through. Proceed with the Isolation RNA Column.

Note: If purification of total nucleic acids including DNA is desired, omit steps 5 and 6 and proceed directly to step 7.

5 Desalt silica membrane:

Add 350 µl Buffer MDB to the Isolation RNA Column (blue ring) and centrifuge for 1 min at 11,000 x g.
Discard flow-through and place the column back into the collection tube.

6 Digest DNA:

Add 100 µl rDNase directly onto the silica membrane of the Isolation RNA Column (blue ring).
Incubate at room temperature (18-25°C) until steps 7–10 are completed, but at least 15 min.

7 Precipitate protein:

Add 300 µl Buffer PPB to the saved flow-through of step 4. Vortex for 5 s.
Centrifuge for 3 min at 11,000 x g to pellet protein.

Note: The protein pellet can be analyzed.

8 Remove residual debris:

Place an Isolation Protein Removal Column (white ring) in a Collection Tube (2 ml, lid) and load the supernatant from step 7 into the column. Centrifuge for 1 min at 11,000 x g.
Discard the Isolation Protein Removal Column and keep the flow–through.

9 Adjust binding conditions for small RNA:

Add 800 µl Buffer BB to the flow–through. Vortex for 5 s.

Note: After addition of Buffer BB a precipitate may be visible. Load all of the precipitate into the column at step 10.

10 Bind small RNA:

Load 600 µl of the mixture from step 9 into the corresponding Isolation RNA Column (blue ring) already containing the large RNA from step 4.
Centrifuge for 30 s at 11,000 x g.

Attention: *Do not centrifuge the Isolation RNA Column with the rDNase Reaction Buffer before the mixture of step 9 is added.*

Discard the flow–through and place the column back into the collection tube.
Repeat this step two times to load the remaining sample.

11 Wash and dry silica membrane:

1st wash

Add 600 µl Buffer WB1 to the Isolation RNA Column.
Centrifuge for 30 s at 11,000 x g.
Discard flow–through and place the column back into the collection tube.

2nd wash

Add 700 µl Buffer WB2 to the Isolation RNA Column.
Centrifuge for 30 s at 11,000 x g.
Discard flow–through and place the column back into the collection tube.

Optionally: Repeat the 2nd wash step to remove trace amounts of chaotropic salt and thus to further increase $A_{260/230}$ ratio.

3rd wash

Add 250 µl Buffer WB2 to the Isolation RNA Column.
Centrifuge for 2 min at 11,000 x g to dry the silica membrane.
Discard Collection Tube and place the Isolation RNA Column into a new Collection Tube (1.5 ml, lid).

12 Elute RNA:

Add 30 µl (high concentration), 50 µl (medium concentration and yield) or 100 ul (high yield) RNase-free H_2O to the Isolation RNA Column.
Incubate for 1 min at room temperature (18-25℃).
Centrifuge for 30 s at 11,000 x g.

13 Use elute RNA and DNA:

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

Purification protocol 2:
RNA purification using phenol-based lysis reagent

1 Cell lysis:

Tissue and plant material

Thoroughly disrupt 50 mg sample material in 500 µl phenol-based lysis reagent using mechanical devices. Sample amount and phenol-based lysis reagent volume can be increased proportionally.
Transfer 500 µl lysate to a Collection Tube (2 ml, lid).

Cultured cells

Cells grown in monolayer:
Remove culture medium and lyse cells by addition of at least 1 ml phenol-based lysis reagent to the culture disk (diameter 3.5 cm, 10 cm^2). Mix by pipetting up and down.

Note: An insufficient volume of phenol-based lysis reagent will lead to DNA contamination of the isolated RNA.

Transfer 500 µl lysate to a Collection Tube (2 ml, lid).
Alternatively, collect up to 10^7 cultured cells after trypsinization by centrifugation, discard supernatant and add 500 µl phenol-based lysis reagent. Pipette up and down or vortex to lyse the cells.

Cells grown in suspension:
Collect up to 10^7 cultured cells by centrifugation, discard supernatant and add 500 µl phenol-based lysis reagent. Pipette up and down or vortex to lyse the cells.

Liquid samples

Use up to 200 µl liquid sample with 500 µl phenol-based lysis reagent.
Incubate 5 min at room temperature (18-25℃).

2 Precipitate contaminants:

Add 200 µl RNase-free H_2O to the lysate in 500 µl phenol-based lysis reagent. Vortex

vigorously and incubate at room temperature for 15 min.
Centrifuge samples for 15 min at 12,000 x g.

Note: DNA, proteins and polysaccharides are pelleted, RNA remains in the supernatant.

Transfer 500 µl supernatant into a fresh Collection Tube (2 ml, lid) without disturbing the pellet. Discard pellet and residual supernatant.

3 Bind RNA

Add 500 µl Buffer BB to the transferred supernatant and mix by vortexing.
Combine an Isolation RNA Column (blue ring) with a Collection Tube (2 ml) and load 500 µl of the sample solution into the column.
Centrifuge for 30 s at 8,000 x g.
Discard flow-through and load the remaining sample.
Centrifuge for 30 s at 8,000 x g and discard flow-through.

4 Wash and dry silica membrane:

1st wash

Add 700 µl Buffer WB2 to the Isolation RNA Column.
Centrifuge for 30 s at 8,000 x g. Discard flow-through.

Optionally: Repeat the 1st wash step to remove trace amounts of chaotropic salt and thus to further increase $A_{260/230}$ ratio.

2nd wash

Add 250 µl Buffer WB2 to the Isolation RNA Column.
Centrifuge for 2 min at 8,000 x g. Discard flow-through.

5 Elute RNA:

Place the Isolation RNA Column in a new Collection Tube (1.5 ml).
Add 30 µl (high concentration), 50 µl (medium concentration and yield) or 100 µl (high yield) RNase-free H_2O to the Isolation RNA Column.
Incubate for 1 min at room temp. (18-25℃).

6 Use elute RNA and DNA: Use fresh isolate samples immediately in downstream applications or freeze isolate samples for later use.

Purification protocol 3:
RNA clean-up

1 Prepare sample: Add 150 µl Buffer LB to 150 µl sample reaction mixture and vortex for 5 s.

Note: To purify less than 150 µl, adjust volume with RNase-free H_2O to 150 µl. To process more than 150 µl, increase volumes of buffers LB, PPB and BB proportionally.

2 Adjust binding conditions for nucleic acids > 200 nt:

Add exactly 200 µl 96–100% ethanol and vortex for 5 s.

Incubate at room temp. (18-25℃) for 5 min.

3 Bind large nucleic acids:

Place an Isolation RNA Column (blue ring) in a Collection Tube (2 ml, lid) and load the sample into the column. Centrifuge for 30 s at 11,000 x g.

Keep the flow-through and the column!

Transfer the Isolation RNA Column containing the large nucleic acids to a new Collection Tube (2 ml) and save it for step 5.

Optional: Digest DNA: Add 350 µl Buffer MDB to the Isolation RNA Column (blue ring) and centrifuge for 1 min at 11,000 x g.

Discard flow-through and place the column back into the collection tube.

Add 100 µl rDNase directly onto the silica membrane of the Isolation RNA Column (blue ring).

Incubate at room temperature (18-25℃) until steps 4 is completed but at least 15 min.

4 Adjust binding conditions for nucleic acids < 200 nt:

Add 100 µl Buffer PPB to the flow-through of step 3 and vortex for 5 s.
Incubate for 5 min at room temperature (18-25℃).
Add 800 µl Buffer BB and vortex for 5 s.

5 Bind small nucleic acids:

Load 700 µl sample into the corresponding Isolation RNA Column containing the large nucleic acids.
Centrifuge for 30 s at 11,000 x g.
Discard flow-through and place the column back into the collection tube.
Repeat this step to load the remaining sample.

6 Wash and dry silica membrane:

1st wash

Add 700 µl Buffer WB2 to the Isolation RNA Column.
Centrifuge for 30 s at 11,000 x g. Discard flow-through.

Optionally: Repeat the 1st wash step to remove trace amounts of chaotropic salt and thus to further increase $A_{260/230}$ ratio.

2nd wash

Add 250 µl Buffer WB2 to the Isolation RNA Column.
Centrifuge for 2 min at 11,000 x g. Discard flow-through.

7 Elute RNA:

Place the Isolation RNA Column in a new Collection Tube (1.5 ml).
Add 30 µl (high concentration), 50 µl (medium concentration and yield) or 100 µl (high yield) RNase-free H_2O to the Isolation RNA Column.
Incubate for 1 min at room temperature (18-25℃).
Centrifuge for 30 s at 11,000 x g.

8 Use elute RNA and DNA: Use fresh isolate samples immediately in downstream applications or freeze isolate samples for later use.

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.

11. TROUBLESHOOTING AND FAQS

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:

30 µl
Volume for **high concentration** but reduced total yield. 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.

50 µl (standard)
Volume for **medium concentration** and total yield. The standard elution buffer volume of 50 µl is sufficient to wet the silica membrane completely. It results a high total yield of miRNA/RNA/DNA and simultaneously maximizes the concentration.

100 µl
Volume for **high yield** but low concentration. 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.

It is possible to **reload the eluate from the first elution step into the column and to use it as elution buffer for a second elution**. Multiple elution steps will increase the total yield.

To increase the yield and the concentration in a single elution step, heat the RNase-free water to 90℃ before elution. However, a high temperature leads to larger pipetting errors and consequently to higher variations in the final volume of the eluate.

Normalization

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

Poor or no RNA yield

Reagents not applied or restored properly

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

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

• Add the indicated volume of 96-100% ethanol to Buffer WB2. 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 to prevent evaporation or contamination.

Sample material not stored properly

• Whenever possible, use fresh material. Otherwise, flash freeze the samples in liquid nitrogen. Samples should always be kept at -70℃. Never allow tissues to thaw before addition of lysis buffer. Perform disruption of samples in liquid nitrogen or lysis buffer.

Insufficient disruption and/or homogenization of starting material

• Ensure thorough sample disruption and use Isolation Filters for homogenization of disrupted starting material.

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.

Clogged isolation RNA Columns

Too much starting material

• Overloading may lead to decreased overall RNA yield due to binding of too much DNA. Reduce amount of sample material or use larger volume of lysis buffer.

Insufficient homogenization of starting material

• After cell lysis in Buffer LB and homogenization with Isolation Filters the lysate has to be clear and free of solid particles. If this is not the case, centrifuge the sample and transfer the clear supernatant to a new collection tube (not provided) without disturbing the pellet.

Too much precipitated nucleic acids after addition of ethanol

• Do not remove the precipitate (e.g. by centrifugation) since it contains large RNA.

• Mix immediately after addition of ethanol to avoid too high local alcohol concentrations.

• Rotate the isolation RNA Column by 180℃ inside t he centrifuge and repeat the loading step as often as necessary until all lysate has completely passed the column.

• Increase centrifugation time and speed to load the sample.

• Use Isolation Filters after ethanol addition to homogenize the lysate.

Clogged Isolation Protein Removal Column

Too much protein precipitate or precipitate too fine

• Pellet the protein by centrifugation before loading the cleared lysate onto the Isolation Protein Removal Column.

• Rotate the Isolation Protein Removal Column by 180℃ inside the centrifuge and repeat the protein removal step.

• Increase centrifugation time and speed and repeat the protein removal step.

Contamination with genomic DNA

Too much cell material used

• Reduce quantity of cells or tissue used.

DNA detection system too sensitive

• Use larger PCR targets (e.g. > 500 bp) or intron spanning primers for RNA analysis.

Suboptimal performance of RNA in downstream experiments

Carry-over of ethanol or salt

• Do not let the flow-through touch the column outlet after the second WB2 wash. Make sure to centrifuge at the corresponding speed for the respective time to remove ethanolic buffer WB2 completely.

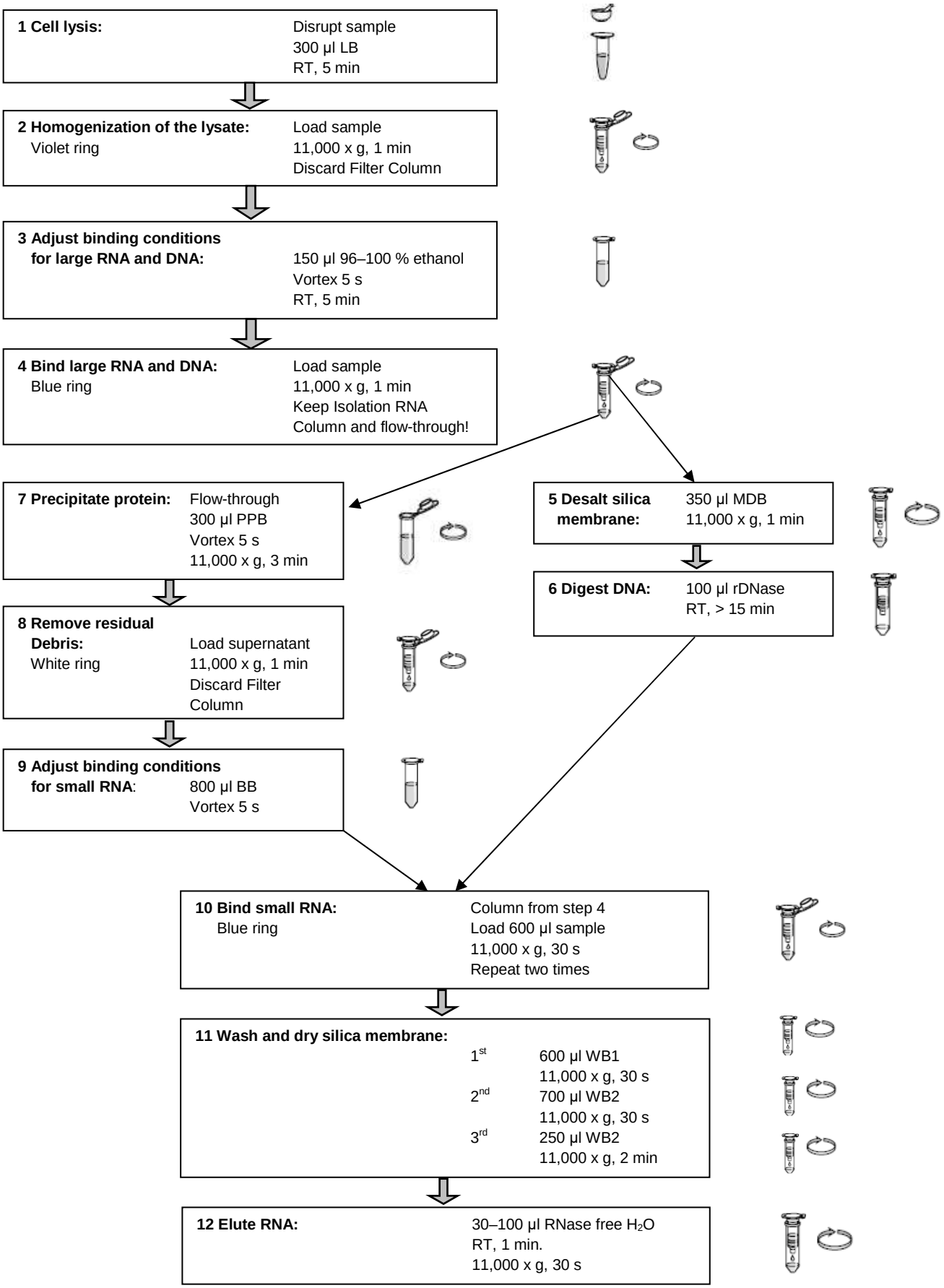
• Check if buffer WB2 has been equilibrated to room temperature (18-25℃) before use. Washing at lower temperatures lowers efficiency of salt removal.

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 1 - summary

RNA purification from human/animal tissue, plant material and cultured cells



Purification Protocol 2 - summary

RNA purification using phenol-based lysis reagent

1 Cell lysis:
300 µl phenol-based lysis reagent
Disrupt sample
RT, 5 min

2 Precipitate contaminants:
200 µl RNase-free H_2O
Vortex 5 s
12,000 x g, 15 min
Transfer 500 µl supernatant into
a fresh Collection Tube (2 ml, lid)

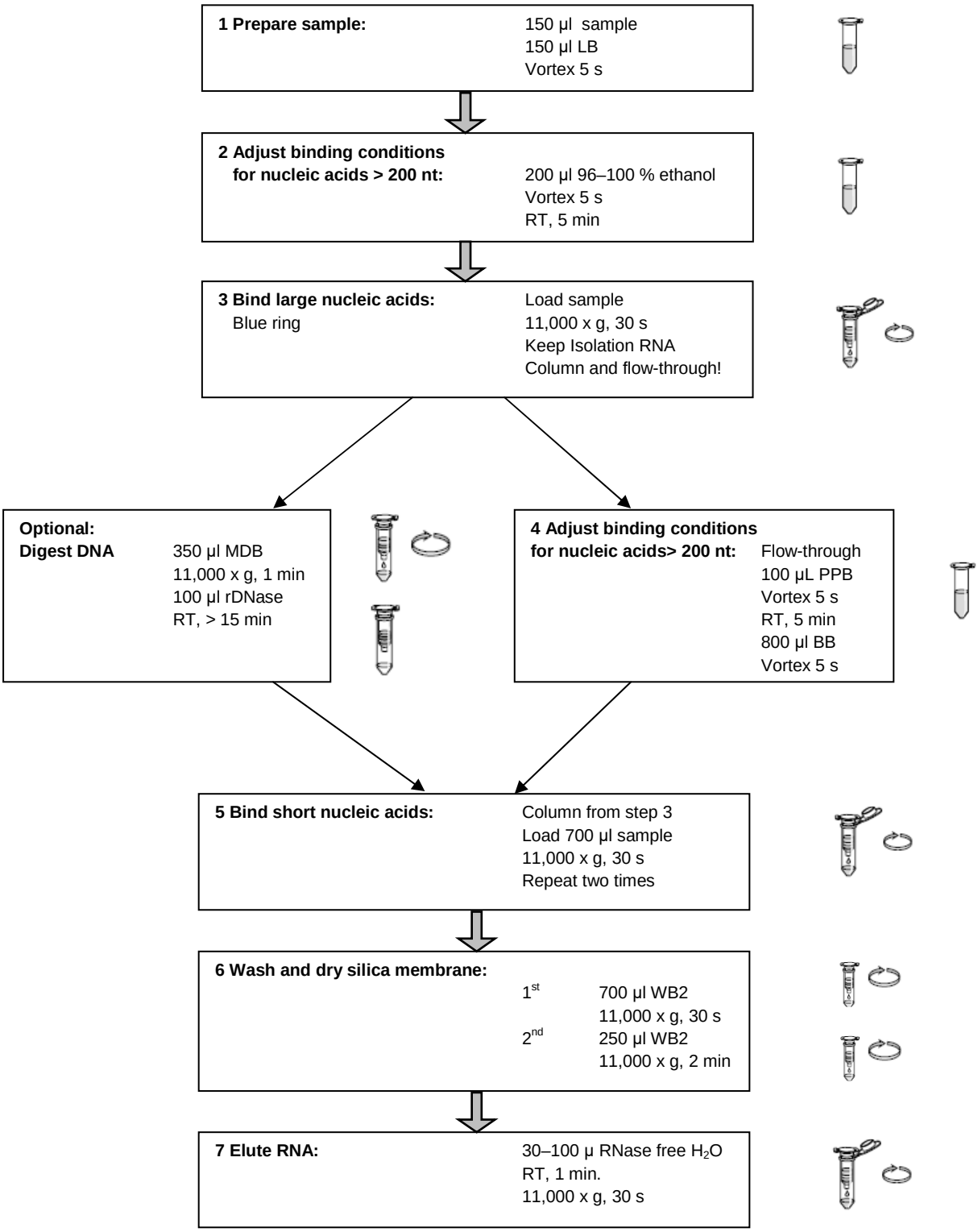
3 Bind RNA:
Blue ring
500 µL BB
Vortex 5 s
Load 500 µl sample
8,000 x g, 30 s
Load remaining sample
8,000 x g, 30 s

4 Wash and dry silica membrane:
1st 700 µl WB2
8,000 x g, 30 s
2nd 250 µl WB2
8,000 x g, 2 min

5 Elute RNA:
30–100 µl RNase free H_2O
RT, 1 min.
11,000 x g, 30 s

Purification Protocol 3 - summary

RNA Clean-up



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