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Blood RNA isolation kit

Cat. No. R-Blood-50

Important!
We are constantly improving the protocol for working with the reagent, so please use the protocol supplied with the product. The latest version of the protocol is available on the website of Biolabmix LLC (www.biolabmix.ru). The kit is intended for research purposes only.
Protocol updated 08/13/2024.

Description

The kit is designed to isolate and purify RNA from the following samples:

1. Whole blood drawn into disposable tubes with EDTA or sodium citrate anticoagulants;

2. Cell Cultures.

The operating principle of the kit is based on selective sorption of nucleic acids on a silicon membrane from a pre-lysed sample, subsequent washing and elution of the purified product.

RNA isolation from whole blood involves selective destruction of erythrocytes with RBC lysis buffer, and leukocytes are precipitated by centrifugation. Leukocytes are efficiently lysed in LB buffer, and RNA integrity is preserved. The lysate is then applied to the first column on which most of the DNA (90% or more) contained in the sample is sorbed. The purified lysate is applied to a second column on which RNA sorption occurs. The last steps of the protocol are followed by washing and elution of the purified product.

RNA elution proceeds in 15-40 µl. Up to 45 µg of RNA can be eluted, depending on the amount and type of sample.

Important: Most of the DNA is removed during the extraction process and no DNAase treatment is required. However, further DNA removal may be necessary for some applications sensitive to very small amounts of DNA.

Table of contents

Protocol 1. RNA isolation from whole blood 5
Protocol 2. RNA isolation from animal cell cultures.......... 8
Protocol 3. RNA isolation from bacterial cell cultures.......... 12
Protocol 4: DNA purification. Optional.......... 15
Analysis of isolated RNA and DNA 16

Set composition

	R-Blood-50 50 extractions
RBC Erythrocyte lysis buffer	5x120 ml
Lysis buffer LB	40 ml
Buffer for sorption BB	60 ml
Wash buffer WB1	60 ml
Wash buffer WB2 (concentrate)	25 ml
Elution buffer EB	15 ml
Spin columns 1D	50 pcs.
Spin columns 2R	50 pcs.

1D columns include filtrate collection tubes and sample sorption columns. Columns with a **larger** filter.
2R columns include filtrate collection tubes and sample sorption columns. Columns with a **smaller** filter.
RBC is also available as a separate item (see **Additional Reagents**).

Precautions

Caution. The lysis buffers LB and wash buffer WB1 contain guanidine thiocyanate solution, which is irritating and toxic. General and personal safety regulations must be observed during work. Toxic in contact with skin and ingestion. Causes burns.
Caution. The sorption buffers BB and rinsing buffer WB1 contain isopropanol, which is irritating and toxic. Do not work with the solution in the immediate vicinity of an open flame.
In case of skin contact, wash immediately with plenty of water and detergent (detergent). Seek medical attention if necessary.
Attention! Disposable rubber gloves should be worn when working with biological fluids, as the material under study is potentially infectious, capable of long-term preservation or transmission of HIV, hepatitis virus or any other viral pathogen.
Disinfect all used materials in accordance with the requirements of Methodological guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices - e.g. MU-287-113.

Operation

Components: RBC, LB, BB, WB1, WB2, EB are stable throughout the shelf life if storage conditions (see on the package) and sufficient sealing of the vials are observed.

Working conditions

Environmental temperature from +15°C to +25°C; Relative air humidity not more than 80%; Atmospheric pressure 630 - 800 mm Hg.

Equipment and materials not included in the kit

- Centrifuge with rotor for 1.5-2 ml microtubes, speed 12000 rcf, cooling to +4°C;

- **Optional:**
  Centrifuge with rotor for at least 15 ml tubes, speed 400 rcf, cooling to +4 °C. For convenience when preparing leukocyte precipitate;
- Vortex;
- Single-channel variable volume pipettes and their tips;
- Rubber gloves;
- 1.5 ml microtubes;
- Ethanol, 96-100% solution;
- 2-mercaptoethanol, 14.3 M solution (commercially available solution usually has a concentration of 14.3 M) or alternatively, an aqueous solution of 2 M dithiothriethol (DTT);

Before starting work

Preparation of LB buffer.

- **Option 1.**

Add 20 µl of 2-mercaptoethanol (2-ME) to 1 ml of LB buffer. Prepare LB buffer with 2-mercaptoethanol on the day of RNA extraction, do not store.

- **Option 2.**

Add 40 µl of 2 M dithiothriethol (DTT) solution to 1 ml of LB buffer. Prepare LB buffer with dithiothriethol (DTT) on the day of RNA extraction, do not store.
Use freshly prepared 2 M DTT solution or single frozen aliquots.
Note: When isolating RNA from blood, always add 2-ME or DTT to the LB buffer. When working with cell cultures, it is possible to work without 2-ME or DTT depending on the cell culture. For example, when working with RNase-rich cell cultures, LB 2-ME or DTT should be added to the buffer.

WB2 buffer preparation.

- **1 wash, 500 µl WB2**.To 100 µl WB2 buffer (concentrate), add 400 µl ethanol (96-100%).
- **R-Blood-50. 1 vial.** Add 100 ml of ethanol (96-100%) to 25 ml of WB2 buffer (concentrate), to obtain 125 ml of WB2 buffer.

Close the lid tightly after adding ethanol. It is recommended that ethanol be added to an aliquot of WB2 buffer, as the ethanol may partially evaporate if the buffer with ethanol is stored for several months.

Protocol 1. RNA isolation from whole blood.

1) Leukocyte preparation

Important: Refrigerate the RBC erythrocyte lysis buffer at +4°C in advance. The use of chilled buffer allows for better RNA preservation during isolation.

1. The tube with whole blood should be mixed gently to avoid stratification of the sample into plasma and cellular fraction. Take up to 1.5 ml of whole blood into a separate tube.
2. Add 5 volumes of chilled RBC lysis buffer to the blood sample from the original blood volume. For example, if 1.5 mL of blood is used, add 7.5 mL of RBC buffer.

Recommendation. In the absence of a centrifuge for tubes of 15 ml or more, lysis of erythrocytes is more convenient in a 15 or 50 ml tube. Then divide the sample into 1.5-2 ml microtubes. If the leukocyte precipitate is subsequently resuspended in RBC or PBS buffer, combine the sample into one tube.

3. Stir gently, turning the test tube over to make sure the solution is homogeneous.
4. Incubate for 15 min at +4 °C. Stir by inverting the tube every 5 min.

5.1. Centrifuge at 400 rcf, 10 min, 4 °C. Completely remove the supernatant without touching the precipitate.

5.2. Resuspend the precipitate in 2 volumes of RBC buffer from the original blood volume. For example, if 1.5 mL of blood is used, use 3 mL of RBC.

5.3. Centrifuge at 400 rcf, 10 min, 4 °C. Completely remove the supernatant without touching the precipitate.

6. Resuspend the cell sediment in PBS by pipetting according to Table 1 (see below).

Note: Ensure that the precipitate is completely resuspended and not on the tube wall. If cells are left as a precipitate on the tube wall, this may reduce the efficiency of cell lysis and result in lower RNA yield.

Table 1: Volumes of PBS for resuspending leukocyte precipitate and LB buffer for leukocyte lysis.

Leukocyte count per ml of blood	PBS	LB
$5*10^6$ cells and less	50 µl	350 µl
$5*10^6$ – $10*10^6$ cells	70 µl	650 µl

2) Leukocyte lysis.

Important: Ensure that 2-ME or DTT is added to the LB lysis buffer (see section **Before you start**)

1. To the leukocyte suspension obtained in section 1 (see **Leukocyte Preparation** section) add an aliquot of LB lysis buffer according to Table 1 (above).

Optional: If the expected RNA yield **is** less than 1-2 µg, add 5 µl (5 mg/mL) of polyA RNA (cat. # polyA-500) during RNA extraction. If polyA RNA is used, it is possible to increase the RNA yield.

Important: If polyA RNA is used, the isolated RNA cannot be analyzed by gel electrophoresis, UV spectrometry, or fluorimetry.

2. Mix the sample thoroughly by pipetting. Do not use a vortex. Ensure that a homogeneous suspension is obtained.
3. Incubate for 10 min at 15-25 ºC.

3) Application to the column. Purification from DNA.

1. Apply no more than 800 µl of lysate to the 1D column. Close the column lid tightly.

Important! The 1D column has a larger diameter filter than the 2R column.

2. Centrifuge for 30 s, 12,000 rcf. Save the filtrate.

Note: If some solution remains on the column after centrifugation, repeat centrifugation by increasing the centrifugation time and/or speed without adding a new sample portion.

Note: If the sample volume is greater than 800 µl, reapply the excess to the same column and repeat centrifugation.

3. Transfer the filtrate to a clean test tube.
4. Column 1D can be discarded.

Optional: column 1D can be used for further purification and elution of DNA (see protocol 4).

Important: If DNA isolation is planned, be sure to familiarize yourself with the protocol before proceeding.

4) Application to the column. RNA sorption.

1. Add an equal volume of BB sorption buffer to the resulting filtrate. For example, if the lysate volume is 400 µl, add 400 µl of BB buffer. Mix thoroughly by pipetting before applying to the column.
2. Apply no more than 800 µl of sample to the 2R column, including any precipitate that may have formed after mixing the lysate with BB buffer. Close the column lid tightly.

Important! The 1D column has a larger diameter filter than the 2R column.

3. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: If some solution remains on the column after centrifugation, repeat centrifugation by increasing the centrifugation time and/or speed without adding a new sample portion.

Note: If the sample volume is greater than 800 µl, reapply the excess to the same column and repeat centrifugation.

5) Column flushing.

1. Apply 500 µl of WB1 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
2. Apply 500 µl of WB2 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: Remember to add ethanol to the WB2 buffer beforehand.

3. Reapply 700 µl of WB2 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
4. Centrifuge the column for 3 min, 12000 rcf to completely remove the WB2 buffer.

6) RNA elution.

1. Transfer the column to a new 1.5-2 mL microtube (not included in the kit). Press the column firmly against the tube.
2. Apply 15 to 40 µl of EB elution buffer to the center of the column filter. Incubate for 2 min at room temperature (15-25 °C). Centrifuge for 2 min, 12000 rcf.
   - **Important:** The recommended elution volume is 20 µl. When eluting with a smaller volume, an aliquot of buffer must be carefully applied to the center of the column filter, otherwise the RNA yield may be reduced.
   - **Important:** If the content of leukocytes in 1 ml of blood is more than $5*10^6$ or the expected RNA yield is more than 20 µg, the recommended elution volume is 30 µl. A smaller elution volume may reduce the RNA yield.
   - The elution buffer is RNase-purified water.
3. Store the eluate containing RNA at -20 °C.

Optional. Most of the DNA is removed during the extraction process and DNAase treatment is not required. However, further DNA removal may be necessary for some applications sensitive to very small amounts of DNA. It is recommended to perform control assays to verify whether the DNA impurity has an effect on the final result.

When using thermolabile DNase (Cat. No. EM-100, EM-250, EM-1250, Biolabmix LLC), it is sufficient to use 0.1 units per 1 µg of RNA solution obtained for complete DNA removal.

Protocol 2. RNA isolation from animal cell cultures.

1) Cell preparation.

-Animal cell cultures. Monolayer cultures.

1. Remove cells from the surface of the culture plate by the method used in the laboratory or by the standard method recommended for that cell culture.
2. Transfer a sample of cell suspension (no more than $10*10^6$ cells) into a disposable microtube.
3. Precipitate the cells by centrifugation for 3 min, 1000 rcf. Carefully collect the supernatant.
4. Resuspend the cell sediment in PBS by pipetting according to Table 2 (see below).

Note: Ensure that the precipitate is completely resuspended and not on the tube wall. If cells are left as a precipitate on the tube wall, this may reduce the efficiency of cell lysis and result in lower RNA yield.

5. Skip to Section 2 (**Cell Lysis**).

Table 2. Volumes of PBS for resuspending leukocyte precipitate and LB buffer for leukocyte lysis.

Number of cells	PBS	LB
$5*10^6$ cells and less	50 µl	350 µl
$5*10^6 – 10*10^6$ cells	70 µl	650 µl

- Animal cell cultures. Monolayer cultures. Culture plates. When working with 12-, 24- or 96-well plates or culture dishes with similar cell area, lysis is allowed directly in the well.

1. Remove the culture medium from the well of the plate
2. In a well of a 12-, 24-, or 96-well plate, add an aliquot of LB lysis buffer according to Table 3 (see below).

Table 2. Volumes of PBS for resuspending leukocyte precipitate and LB buffer for leukocyte lysis.

Plate	LB
12-well	400 µl
24-well	200 µl
96-well	200 µl

3. Incubate for 10 minutes. Carefully, avoiding foaming, mix the well contents by pipetting, make sure that the cells have detached from the cell.

4. With a clean disposable tip, transfer the sample into a disposable microtube.
6. Skip to Section 3 (**Application to column. DNA purification**).

–Animal cell cultures. Suspension cultures.

1. Transfer a sample of cell suspension (no more than 2-3*10^6 cells) into a disposable microtube.
2. Precipitate the cells by centrifugation for 3 min, 1000 rcf. Carefully collect the supernatant.
3. Resuspend the cell sediment in PBS by pipetting according to Table 2 (see above).

Note: Ensure that the precipitate is completely resuspended and not on the tube wall. If cells are left as a precipitate on the tube wall, this may reduce the efficiency of cell lysis and result in lower RNA yield.

4. Skip to the next section (Section 2, **Cell Lysis**).

2) Cell lysis.

1. To the cell suspension, add an aliquot of LB lysis buffer according to Table 2 (see above).

Optional: If the expected RNA yield **is** less than 1-2 µg, add 5 µl (5 mg/mL) of polyA RNA (cat. no. polyA-500) during RNA extraction. If polyA RNA is used, it is possible to increase the RNA yield.

Important: If polyA RNA is used, the isolated RNA cannot be analyzed by gel electrophoresis, UV spectrometry, or fluorimetry.

2. Mix the sample thoroughly by pipetting. Do not use a vortex. Ensure that a homogeneous suspension is obtained.
3. Incubate for 10 min at 15-25 °C.

2) Application to the column. Purification from DNA.

1. Apply no more than 800 µl of lysate to the 1D column. Close the column lid tightly.

Important! Not to be confused, the 1D column has a larger diameter filter than the 2R column.

2. Centrifuge for 30 s, 12,000 rcf. Save the filtrate.

Note: If some solution remains on the column after centrifugation, repeat centrifugationby increasing the centrifugation time and/or speed without adding a new sample portion.

Note: If the sample volume is greater than 800 µl, reapply the excess to the same column and repeat centrifugation.

3. Transfer the filtrate to a clean test tube.
4. Column 1D can be discarded.

Optional: column 1D can be used for further purification and elution of DNA (see protocol 4).

Important: If DNA isolation is planned, be sure to familiarize yourself with the protocol before proceeding.

3) Application to the column. RNA sorption.

1. Add an equal volume of BB sorption buffer to the resulting filtrate. For example, if the lysate volume is 400 μl, add 400 μl of BB buffer. Mix thoroughly by pipetting before applying to the column.
2. Apply no more than 800 μl of sample to the 2R column, including any precipitate that may have formed after mixing the lysate with BB buffer. Close the column lid tightly.

Important! The 1D column has a larger diameter filter than the 2R column.

3. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: If some solution remains on the column after centrifugation, repeat centrifugation by increasing the centrifugation time and/or speed without adding a new sample portion.

Note: If the sample volume is greater than 800 μl, reapply the excess to the same column and repeat centrifugation.

4) Column flushing.

1. Apply 500 μl of WB1 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
2. Apply 500 μl of WB2 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: Remember to add ethanol to the WB2 buffer beforehand.

3. Reapply 500 μl of WB2 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
4. Centrifuge the column for 3 min, 12000 rcf to completely remove the WB2 buffer.

5) RNA elution.

1. Transfer the column to a new 1.5-2 mL microtube (not included in the kit). Press the column firmly against the tube.
2. Apply 15 to 40 μl of EB elution buffer to the center of the column filter. Incubate for 2 min at room temperature (15-25 °C). Centrifuge for 2 min, 12000 rcf.
   - **Important:** The recommended elution volume is 20 μl. When eluting with a smaller volume, an aliquot of buffer must be carefully applied to the center of the column filter, otherwise the RNA yield may be reduced.
   - **Important!** When working with $5*10^6$ cells or more or the expected RNA yield is more than 20 μg, the recommended elution volume is 30 μl. A lower elution volume may result in a lower RNA yield.

- The elution buffer is RNase-purified water.

3. Store the eluate containing RNA at -20 °C.

Optional. Most of the DNA is removed during the extraction process and DNAase treatment is not required. However, further DNA removal may be necessary for some applications sensitive to very small amounts of DNA. It is recommended to perform control assays to verify whether the DNA impurity has an effect on the final result.

When using thermolabile DNase (Cat. No. EM-100, EM-250, EM-1250, Biolabmix LLC), it is sufficient to use 0.1 units per 1 µg of RNA solution obtained for complete DNA removal.

Protocol 3. RNA isolation from bacterial cell cultures.

1) Cell preparation.

-Bacterial cell cultures. Gram-negative bacteria.

1. Transfer 0.5-2 ml of overnight cell culture suspension (no more than $1*10^8$ cells) into a disposable microtube.
2. Precipitate the cells by centrifugation for 1 min, 10000 rcf. Carefully collect the supernatant.
3. Resuspend the cell sediment in 50 µl PBS by pipetting.

Note: Ensure that the precipitate is completely resuspended and not on the tube wall. If cells are left as a precipitate on the tube wall, this may reduce the efficiency of cell lysis and result in lower RNA yield.

4. Skip to the next section (Section 2, **Cell Lysis**).

-Bacterial cell cultures. Gram-positive bacteria. Preparation of lysozyme solution:

- Prepare buffer to dissolve the lysozyme in advance
  - 50 mM Tris-HCl (pH 8), 10 mM EDTA (pH 8), 50% glycerol
    Note: The lysozyme solution can be stored in this buffer for at least 6 months at -20 °C.
  - 50 mM Tris-HCl (pH 8), 10 mM EDTA (pH 8)
    Note: The lysozyme solution can be stored in this buffer for a maximum of 1 week at +4 °C.
- To the lysozyme suspension with a clean disposable tip, add the required volume of lysozyme dissolution buffer to obtain a 50 mg/mL solution.
- Stir thoroughly on the vortex.
- Incubate for 30 min at Tkomn (15-25° C), stirring occasionally until lysozyme is completely dissolved.

1. Transfer 0.5-2 ml of overnight cell culture suspension (no more than $1*10^8$ cells) into a disposable microtube.
2. Precipitate the cells by centrifugation for 1 min, 10000 rcf. Carefully collect the supernatant.
3. Resuspend the cell sediment in 50 µl PBS by pipetting.

Note: Ensure that the precipitate is completely resuspended and not on the tube wall. If cells are left as a precipitate on the tube wall, this may reduce the efficiency of cell lysis and result in lower RNA yield.

4. With a clean disposable tip, add 30 µl of lysozyme solution (50 mg/mL).

Note: lysozyme is not included in the kit.

5. Stir the sample on the vortex for 5-10 s. Reset the droplets by short centrifugation. Incubate for 10 min at Tcomn (15-25 °C).
6. Skip to the next section (Section 2, **Cell Lysis**).

2) Cell lysis.

1. Add 650 µl of LB lysis buffer to the cell suspension.

Optional: If the expected RNA yield **is** less than 1-2 µg, add 5 µl (5 mg/mL) of polyA RNA (cat.# polyA-500) during RNA extraction. It is possible to increase the RNA yield if polyA RNA is used.

Important: If polyA RNA is used, the isolated RNA cannot be analyzed by gel electrophoresis, UV spectrometry, or fluorimetry.

2. Mix the sample thoroughly by pipetting. Do not use a vortex. Ensure that a homogeneous suspension is obtained.
3. Incubate for 10 min at 15-25°C.

2) Application to the column. Purification from DNA.

1. Apply no more than 800 µl of lysate to the 1D column. Close the column lid tightly.

Important! The 1D column has a larger diameter filter than the 2R column.

2. Centrifuge for 30 s, 12,000 rcf. Save the filtrate.

Note: If some solution remains on the column after centrifugation, repeat centrifugation by increasing the centrifugation time and/or speed without adding a new sample portion.

Note: If the sample volume is greater than 800 µl, reapply the excess to the same column and repeat centrifugation.

3. Transfer the filtrate to a clean test tube.
4. Column 1D can be discarded.

Optional: column 1D can be used for further purification and elution of DNA (see protocol 4).

Important: If DNA isolation is planned, be sure to familiarize yourself with the protocol before proceeding.

3) Application to the column. RNA sorption.

1. Add an equal volume of BB sorption buffer to the resulting filtrate. For example, if the lysate volume is 400 µl, add 400 µl of BB buffer. Mix thoroughly by pipetting before applying to the column.
2. Apply no more than 800 µl of sample to the 2R column, including any precipitate that may have formed after mixing the lysate with BB buffer. Close the column lid tightly.

Important! The 1D column has a larger diameter filter than the 2R column.

3. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: If some solution remains on the column after centrifugation, repeat centrifugation by increasing the centrifugation time and/or speed without adding a new sample portion.

Note: If the sample volume is greater than 800 µl, reapply the excess to the same column and repeat centrifugation.

4) Column flushing.

1. Apply 500 µl of WB1 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
2. Apply 500 µl of WB2 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: Remember to add ethanol to the WB2 buffer beforehand.

3. Reapply 500 µl of WB2 wash buffer to the column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
4. Centrifuge the column for 3 min, 12000 rcf to completely remove the WB2 buffer.

5) RNA elution.

1. Transfer the column to a new 1.5-2 mL microtube (not included in the kit). Press the column firmly against the tube.
2. Apply 15 to 40 µl of EB elution buffer to the center of the column filter. Incubate for 2 min at room temperature (15-25 °C). Centrifuge for 2 min, 12000 rcf.
   - **Important:** The recommended elution volume is 20 µl. When eluting with a smaller volume, an aliquot of buffer must be carefully applied to the center of the column filter, otherwise the RNA yield may be reduced.
   - **Important:** If the expected RNA yield is greater than 20 µg, the recommended elution volume is 30 µl. A smaller elution volume may reduce the RNA yield.
   - The elution buffer is RNase-purified water.
3. Store the eluate containing RNA at -20 °C.

Optional. Most of the DNA is removed during the extraction process and DNAase treatment is not required. However, further DNA removal may be necessary for some applications sensitive to very small amounts of DNA. It is recommended to perform control assays to verify whether the DNA impurity has an effect on the final result.

When using thermolabile DNase (Cat. No. EM-100, EM-250, EM-1250, Biolabmix LLC), it is sufficient to use 0.1 units per 1 µg of RNA solution obtained for complete DNA removal.

Protocol 4: DNA purification. Optional.

Note: The following is the protocol for purification and elution of DNA from column 1D. If you plan to isolate DNA in parallel with RNA, please familiarize yourself with this protocol in advance. Washing and elution of columns 1D (for DNA extraction) and 1R (for RNA extraction) can be performed simultaneously.

Important: Store the 1D column after DNA sorption and before washing for no more than 30 min at 2-25 °C.

1) 1D column flushing.

1. Apply 500 µl of WB1 wash buffer to the 1D column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
2. Apply 500 µl of WB2 wash buffer to the 1D column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.

Note: Remember to add ethanol to the WB2 buffer beforehand.

3. Reapply 500 µl of WB2 wash buffer to the 1D column. Centrifuge for 30 s, 12000 rcf. Remove the filtrate.
4. Centrifuge the column for 3 min, 12000 rcf to completely remove the WB2 buffer.

2) DNA elution.

1. Transfer the column to a new 1.5-2 mL microtube (not included in the kit). Press the column firmly against the tube.
2. Apply 60 to 100 µl of EB elution buffer to the center of the column filter. Incubate for 3 min at room temperature (15-25°C). Centrifuge for 2 min, 12000 rcf.
   - **Important:** The recommended elution volume is 100 µl. If a smaller elution volume is used, an aliquot of buffer must be carefully applied to the center of the column filter, otherwise the DNA yield may be reduced.
   - The elution buffer is RNase-purified water.
3. Store the eluate containing DNA at -20°C.

Analysis of isolated RNA and DNA

The integrity of isolated RNA and DNA can be verified by gel electrophoresis in 1% agarose gel.
The amount of isolated RNA and DNA can be estimated by UV spectrometry. The characteristic absorption maximum for RNA and DNA is at λ = 260 nm.
Calculate the RNA concentration (μg/mL) using the following formula:
A_{260} * dilution * 40 μg/mL.
Calculate the RNA concentration (μg/mL) using the following formula: A_{260} * dilution * 50 μg/mL.
Characteristic A_{260}/A_{280} optical density ratios are ~1.8 to 2.0.

Note: If polyA RNA was used in the isolation process, the isolated RNA and DNA cannot be analyzed by gel electrophoresis, UV spectrometry, or fluorimetry. PCR is recommended for analysis.

Additional reagents:

- RBC red blood cell lysis buffer (Cat. No. RBC-100, RBC-500).
- PolyA solution, 5 mg/mL (Cat. No. polyA-500).
- Thermolabile DNAase, 2 units/μL (Cat. No. EM-100, EM-250, EM-1250).
- Kit for isolation of DNA and RNA from reaction mixtures (Cat. No. DR-10, DR-50, DR-250).
- Buffers for agarose gel electrophoresis: Tris-acetate buffer (Cat. No. BE-DNA-500, BE-DNA-1000), Tris-borate buffer (Cat. No. TBE-500).
- Ethidium bromide solution for NK visualization (Cat. No. EtBr-10).
- Buffers for introducing DNA and RNA samples into the gel (Cat. No. D-3001, D-3002, D-3003).

Storage conditions:

Store the kit for RNA isolation at a temperature of +15 °C to +25 °C. For expiration date, see the package.

Transportation Conditions:

The set should be transported at temperatures between +15°C and +25°C.