Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific
GeneJET Stabilized and Fresh Whole Blood RNA Kit

#K0871

Read storage information (p.4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

#K0871

Lot __

Expiry Date __

CERTIFICATE OF ANALYSIS

Thermo Scientific™ GeneJET™ Stabilized and Fresh Whole Blood RNA Kit is qualified by isolating total RNA from 250 µL of human whole blood following the protocol outlined in the manual. The quality of isolated RNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified RNA has an A_{260}/A_{280} ratio between 1.9 and 2.1 and the RNA integrity number (RIN) of ≥ 8.

Quality authorized by:  Jurgita Žilinskienė

Rev.1 . 69

Contents **page**

COMPONENTS OF THE KIT

GeneJET Stabilized and Fresh Whole Blood RNA Kit	#K0871 50 preps
Lysis Buffer for GeneJET Blood RNA Kit	40 mL
Wash Buffer 1 (conc.) for GeneJET Blood RNA Kit	24 mL
Wash Buffer 2 (conc.) for GeneJET Blood RNA Kit	25 mL
DNase I (lyophilized)	1 vial
DNase I Reconstitution Buffer	1 mL
2X DNase I Buffer	4 mL
Manganese Chloride Solution	1 mL
Water, nuclease-free	30 mL
GeneJET RNA Purification Columns pre-assembled with Collection Tubes	50
Collection Tubes, 2 mL	3 × 50
Collection Tubes, 1.5 mL	50

STORAGE

When the kit is delivered, remove DNase I (lyophilized), 2X DNase Buffer, DNase I Reconstitution Buffer and Manganese Chloride Solution and store at -20 °C. Reconstituted DNase I should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).
Note. Close the bag with GeneJET RNA Purification columns tightly after each use!

DESCRIPTION

The GeneJET Whole Blood RNA Kit is designed for rapid and efficient purification of high quality total RNA from various whole blood samples: fresh, frozen, or human blood samples stabilized with commercial stabilizers such as Tempus™ and PAXgene™.

The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The kit includes a DNase I step for the enzymatic removal of genomic DNA. RNA can be isolated from whole blood in less than 30 minutes (including cell lysis and DNase I treatment). The purified high quality RNA can be used in a wide range of downstream applications such as RT-PCR, RT-qPCR, Northern blotting and other RNA-based analysis.

PRINCIPLE

Blood is collected into vials and stabilized by anticoagulants. Cells are lysed in a buffer containing guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases. The lysate is then mixed with ethanol and loaded on the purification column. The chaotropic salt and ethanol facilitate RNA binding to the silica membrane when the lysate is spun through the column. Contaminating DNA, which is also bound to the silica membrane, is removed by a DNase I solution which is directly applied onto the silica membrane. Subsequently, impurities are effectively removed by treating the column with the provided wash buffers. Pure RNA is then eluted under low ionic strength conditions with the provided nuclease-free water.

Table 1. Typical total RNA yields per 1 mL whole blood from different species.

Source	Yield, µg
Human blood	4-8 (up to 12)
Mouse blood	up to 135
Rabbit blood	up to 25
Cattle blood	2-8
Buffy coat	2-3

IMPORTANT NOTES

- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to first use:

	#K0871 50 preps	
	Wash Buffer 1	**Wash Buffer 2**
Concentrated wash solution	24 mL	25 mL
Ethanol (96-100%)	**64 mL**	**100 mL**
Total volume:	88 mL	125 mL

After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- To prepare the **DNase I solution** add 0.44 mL of **DNase I Reconstitution Buffer** to each vial of **DNase I (lyophilized)** and incubate at room temperature for 5 minutes. Occasional gentle rotation of the vial helps to dissolve the DNase I, but avoid forceful mixing. Do not vortex! Store at -20 °C.
- Check the **Lysis Buffer** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37 °C, then cool back down to 25 °C before use.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution**. Add 20 µL of 2 M DTT to each 1 mL of Lysis Buffer used.
- Wear gloves when handling the **Lysis Buffer and Wash Buffer 1** as these solutions contain irritants (*see* p. 18 for SAFETY INFORMATION) and are harmful if they come into contact with skin, inhaled or swallowed.
- Unless otherwise indicated, all purification steps are performed at room temperature (15-25 °C).
- Centrifugation speed (rpm) is given for 24-place microcentrifuges.
- Typically the purified RNA has an A_{260}/A_{280} ratio between 1.9 and 2.1, however, when RNA concentration is lower than 20 ng/µL, deviations from the expected ratio are occasionally observed.
- Kit will remove the majority of genomic DNA, however trace amounts may still remain.Therefore for very sensitive for genomic DNA contamination applications we recommend to treat the purified RNA sample with DNase I (#EN0521).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 2 M DTT (dithiotreitol) solution (store at -20 °C)
- 96-100% ethanol, molecular biology grade
- Pipettes and RNase-free pipette tips
- Vortex
- RNase free 1.5 or 2 mL plastic tubes
- Centrifuge capable of ≥ 20,000 × g for microtubes
- Centrifuge capable of 3,000-4,000 × g with swinging-bucket rotor
- Disposable gloves
- RBC buffer (Red Blood Cells Lysis buffer)
- 15 mL sterile, polypropylene tubes for erythrocyte lysis.

AVOIDING RIBONUCLEASE CONTAMINATION

RNA purity and integrity is essential for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNases into the RNA preparation, especially during the column wash and RNA elution steps. General recommendations to avoid RNase contamination include:

- As skin is a common source of RNases, wear gloves when handling reagents and RNA samples. Change gloves frequently.
- Use sterile, disposable RNase-free pipette tips.
- Use reagents designed to remove RNase contamination from nondisposable items (pipettes, centrifuges) and work surfaces.
- Keep all kit components tightly sealed when not in use. After usage, cap bottles immediately.

STARTING MATERIAL HANDLING AND STORAGE

- **Blood sample collection and RNA purification from fresh blood cells should be carried out in the same day**. Samples can be stored at 4 °C for no longer than 5 hours (when EDTA, citrate, or heparin collected tubes are used).
- If it is not possible to process samples on the same day, RNA can be preserved in the supplied Lysis Buffer (supplemented with DTT). Aliquot the fresh blood (250 µL or 500 µL) and add 1 volume of Lysis Buffer. Mix well by vortexing.
  Samples preserved with Lysis Buffer can be stored for 48 h at 4 °C.
- If frozen blood samples must be processed, aliquot fresh blood (250 µL each) and store at -70 °C. Add 250 µL of Lysis Buffer (supplemented with DTT) to 250 µL of frozen blood and mix immediately by vortexing until the sample is fully thawed.
- Storage conditions for PAXgene stabilized blood: up to 3 days at room temperature (15-25 °C); up to 5 days at 2-8 °C temperature; long-term storage at -20 to -80 °C.
  Note: After collection of blood sample, it is important to incubate the PAXgene Blood RNA Tube for at least 2 hours at room temperature (15-20 °C) before RNA purification. Overnight incubation may increase RNA yields in some cases. If blood samples in PAXgene Blood RNA Tubes were frozen, they must be thawed at room temperature for at least 2 hours before RNA purification.
- Storage conditions for Tempus stabilized blood: up to 5 days at room temperature, up to 7 days at 4 °C temperature; long-term storage at -20 to -80 °C. If the sample is frozen, thaw the sample in the Tempus tube for 10 to 30 minutes on ice.

PROTOCOL SELECTION GUIDE

The GeneJET Stabilized and Fresh Whole Blood RNA Kit provides optimized protocols for total RNA purification from different amounts and types of starting material.
The following section guide summarizes available protocols depending on starting sample volume and sample processing type.

Sample type	Sample volume	Protocol	Page
Fresh whole blood	**250 µL**	**Protocol A**	page 8
	500 µL	**Protocol B**	page 10
Frozen whole blood	**250 µL**	**Protocol C**	page 11
Blood erythrocyte mass	**250-500 µL of whole blood**	**Protocol D**	page 12
Blood leucocytes	**0.5-1.50 mL of whole blood**	**Protocol E**	page 13
PAXgene stabilized blood	**1-2 mL**	**Protocol F**	page 14
Tempus stabilized blood	**1-2 mL**	**Protocol G**	Page 15
Buffy Coat	**10 mL whole blood**	**Protocol H**	Page 16

PROTOCOLS

Protocol A. Total RNA Purification Protocol from 250 µL of fresh whole blood

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.

<table>
<tr><th>Step</th><th>Procedure</th></tr>
<tr><td>1</td><td>Transfer 250 µL of whole blood into a 2 mL microcentrifuge tube (not included).</td></tr>
<tr><td>2</td><td>Add 250 µL of Lysis Buffer (supplemented with DTT), mix well by vortexing. Incubate at room temperature (15-25 °C) for 5-6 minutes to lyse the blood cells. Mix by vortexing briefly several times during incubation. Invert the tube to be sure the solution is homogeneous.
Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.</td></tr>
<tr><td>3</td><td>Add 250 µL of ethanol (96-100%) and mix by pipetting or vortexing.
Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.</td></tr>
<tr><td>4</td><td>Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included).
Note. Close the bag with GeneJET RNA Purification columns tightly after each use!</td></tr>
<tr><td>5</td><td>Add 700 µL of Wash Buffer 1 (supplemented with ethanol). Centrifuge for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column into a new 2 mL collection tube (included).</td></tr>
<tr><td>6</td><td>Add 700 µL of Wash Buffer 2 (supplemented with ethanol) to the purification column. Centrifuge for 1 min at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column back into the collection tube.</td></tr>
<tr><td>7</td><td>Prepare DNase I Master Mix for the number of samples used for purification.

DNase I Master Mix for purification of one sample (100 µL):
<table>
<tr><th>Component</th><th>Quantity, for 1 RNA prep</th></tr>
<tr><td>2X DNase I Buffer</td><td>50 µL</td></tr>
<tr><td>DNase I (reconstituted)</td><td>8 µL</td></tr>
<tr><td>Manganese Chloride Solution</td><td>10 µL</td></tr>
<tr><td>Water, nuclease-free</td><td>32 µL</td></tr>
</table>
Mix all components properly.</td></tr>
<tr><td>8</td><td>Add 100 µL DNase I Master Mix onto the column. Incubate at room temperature for 15 min. Do not centrifuge after incubation.</td></tr>
</table>

9	Add 500 µL of **Wash Buffer 1** (supplemented with ethanol) to the purification column. Centrifuge for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column back into the collection tube.
10	Add 700 µL of **Wash Buffer 2** (supplemented with ethanol) to the purification column. Centrifuge for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column back into the collection tube.
11	Add 500 µL of **Wash Buffer 2** (supplemented with ethanol) to the purification column. Centrifuge for 1 min at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place column into a new 2 mL collection tube (included). Centrifuge the column for 1 min at maximum speed (≥ 20,000 × g, ≥ 14,000 rpm). Discard the collection tube containing the flow-through solution and transfer the purification column to an RNase-free 1.5 mL microcentrifuge tube.
12	Add 50 µL of **nuclease-free water** to the center of the purification column membrane and centrifuge for 1 min at 12,000 × g (~ 11,000 rpm).
13	Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20 °C or -70 °C until use. Keep the RNA on ice after extraction and while working with it. **Note.** For prolonged storage (more than 1 month) storage at -70 °C is recommended.

Protocol B. Total RNA Purification Protocol from 500 µL of fresh whole blood

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.

Step	Procedure
1	Transfer 500 µL of whole blood in a 2 mL microcentrifuge tubes (not included).
2	Add 500 µL of **Lysis Buffer** (supplemented with DTT), mix well by vortexing. Incubate at room temperature (15-25 °C) for 5-6 minutes to lyse the blood cells. Mix by vortexing briefly several times during incubation. Invert the tube to be sure the solution is homogeneous. Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.
3	Add 500 µL of ethanol (96-100%) and mix by pipetting or vortexing. Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.
4	Transfer half (750 µL) of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column back into the collection tube. Transfer the remaining lysate (750 µL) into the column and centrifuge as described above. Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included). **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
5	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

Protocol C. Total RNA Purification Protocol from 250 µL of frozen whole blood

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.

Step	Procedure
1	Add 250 µL of **Lysis Buffer** (supplemented with DTT) to 250 µL of frozen blood sample and thaw immediately by vortexing until the sample is fully thawed. Incubate at room temperature (15-25 °C) for 5-6 minutes. Mix by vortexing briefly several times during incubation. Invert the tube to be sure the solution is homogeneous. Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.
2	Add 250 µL of ethanol (96-100%) and mix by pipetting or vortexing. Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.
3	Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included). **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
4	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

Protocol D. Total RNA Purification Protocol from blood erythrocyte mass

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.

Step	Procedure
1	Centrifuge 250 µL or 500 µL of fresh whole blood for 5 min, 400 × g (~ 2,000 rpm) at 4 °C. Discard the supernatant.
2	Resuspend the pellet in 1 volume of **Lysis Buffer** (supplemented with DTT): • For example, for 250 µL of blood add 250 µL Lysis Buffer, for 500 µL of blood add 500 µL Lysis Buffer, etc. **Mix well** by vortexing. Incubate at room temperature (15-25 °C) for 5-6 minutes. Mix by vortexing briefly several times during incubation. Invert the tube to be sure the solution is homogeneous. Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.
3	Add ethanol (96-100%) and mix by pipetting or vortexing: • If 250 µL of blood is used for purification, add 250 µL of ethanol • If 500 µL of blood is used for purification, add 500 µL of ethanol, etc. Centrifuge for 2 s at 1,000-2,000 × g to clean the lid.
4	Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included). If lysate volume is more than 750 µL transfer remaining lysate into the column and repeat the centrifugation step. **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
5	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

Protocol E. Total RNA Purification Protocol from blood leukocytes

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.
- Prepare 10X RBC Buffer: 1.68 M NH_4Cl with 2 mM EDTA. Dilute with nuclease-free water to 1X concentration.

Step	Procedure
1	Mix 1 volume (0.5-1.5 mL) of whole blood with 5 volumes of 4 °C prepared 1x RBC buffer in a 15 mL sterile, polypropylene tube (not included).
2	Mix completely by vortexing and incubate on ice for 10-15 minutes, mixing briefly by vortexing two times during incubation. The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes.
3	Centrifuge at 1,700 × g (using a swing-out rotor) at 4 °C for 5 minutes, then carefully remove and discard the supernatant completely without disturbing the visible, white leukocyte pellet.
4	Resuspend the leukocyte pellet in 2 volumes of 4 °C prepared 1x RBC buffer. Use 2 volumes of 1x RBC buffer per volume of whole blood used in step 1.
5	Centrifuge at 1,700 × g at 4 °C for 5 minutes, then carefully remove and discard the supernatant completely without disturbing the visible, white leukocyte pellet.
6	Add 500 µL of **Lysis Buffer** (supplemented with DTT), mix well by vortexing. Incubate at room temperature (15-25 °C) for 5 minutes. Mix by vortexing briefly several times during incubation.
7	Add 250 µL of ethanol (96-100%) and mix by pipetting or vortexing.
8	Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column into a new 2 mL collection tube (included). **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
9	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

Protocol F. Total RNA Purification Protocol from PAXgene stabilized whole blood

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.
- According to manufacturer's recommendations, a typical stabilized blood sample volume in PAXgene® Blood RNA Tube is 9.4 mL (6.9 mL Additive + 2.5 mL whole blood). For RNA purification per one GeneJET column we recommend the use of 1 -2 mL of PAXgene blood lysate.

Step	Procedure
1	Invert the PAXgene® Blood RNA Tube several times to be sure the blood lysate is homogeneous. Transfer 1-2 mL of prepared PAXgene blood lysate to plastic tube and centrifuge at 3,000-5,000 × g for 10 minutes at room temperature (15-25 °C) to pellet the crude RNA.
2	Carefully discard the supernatant from each tube and wash the pellet by adding 1 mL of nuclease-free water. Vortex for 20-30 s and collect the pellet by centrifugation at 3,000-5,000 × g for 10 minutes.
3	Carefully discard the supernatant, add 100 µL of nuclease-free water, and vortex for 10-20 s to resuspend precipitates.
4	Add 400 µL of **Lysis Buffer** (supplemented with DTT), mix well by vortexing. Incubate at room temperature (15-25 °C) for 5 minutes. Mix by vortexing briefly several times during incubation.
5	Add 250 µL of ethanol (96-100%) and mix by pipetting or vortexing.
6	Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column into a new 2 mL collection tube (included). **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
7	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

Protocol G. Total RNA Purification Protocol from from Tempus stabilized whole blood

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.
- According to manufacturer's recommendations, a typical stabilized blood sample volume in a Tempus™ Blood RNA Tube is 9 mL (6 mL RNA stabilization solution + 3 mL whole blood). For RNA purification per one GeneJET column we recommended the use of 1 -2 mL of Tempus blood lysate.

Step	Procedure
1	Invert the Tempus™ Blood RNA Tube several times to be sure the blood lysate is homogeneous. Transfer 1-2 mL of prepared Tempus blood lysate to plastic tube and centrifuge at maximum speed (12,000-16,000 × g) for 10 minutes at 4 °C to pellet the crude RNA.
2	Carefully discard the supernatant from each tube. **Note:** the crude RNA pellet in each tube is transparent and invisible. Handle each tube carefully so that you do not dislodge the crude RNA pellet from the tube bottom.
3	Add 500 µL of **Lysis Buffer** (supplemented with DTT); mix well by vortexing to reusupend the pellet. Incubate at room temperature (15-25 °C) for 5 minutes. Mix by vortexing briefly several times during incubation.
5	Add 250 µL of ethanol (96-100%) and mix by pipetting or vortexing.
6	Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column into a new 2 mL collection tube (included). **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
7	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

Protocol H. RNA Purification from buffy coat

Note:

- When using the GeneJET Stabilized and Fresh Whole Blood RNA Kit for the first time, prepare working solutions of **Wash Buffer 1**, **Wash Buffer 2** and reconstitute **DNase I (lyophilized)** as described on page 5.
- Before each RNA purification, prepare a fresh aliquot of **Lysis Buffer** supplemented with **2 M DTT solution** as described on page 5 and ensure that 2X DNase I Buffer and Manganese Chloride are thawed.
- Prepare 10X RBC Buffer: 1.68 M NH_4Cl with 2 mM EDTA. Dilute with nuclease-free water to 1X concentration.
- Buffy coat is a leukocyte-enriched fraction of whole blood and contains approximately 5-10 times more nucleic acids than an equivalent volume of whole blood.

Step	Procedure
1	Centrifuge 10 mL of whole blood at 2,000 × g for 10 minutes at room temperature. **Note**. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat (also called the WBCs), containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.
2	Remove upper clear layer by aspiration, be careful not to disturb the intermediate layer - buffy coat.
3	Collect approximately **500** µL of the intermediate layer using an automatic pipette, being careful not to disturb the WBCs. Put the WBCs into a fresh tube.
4	Add 10 mL of prepared 1x RBC (red blood cell) buffer and resuspend WBCs.
5	Mix by vortexing and incubate on ice for 5-10 minutes. Mix briefly by vortexing two times during incubation.
6	Centrifuge at 2,000 × g for 10 minutes at room temperature. Remove supernatant. **Do not disrupt the pellet**.
7	Add 500 µL of **Lysis Buffer** (supplemented with DTT); **mix well** by vortexing to reusupend the pellet. Incubate at room temperature (15-25 °C) **for 5 minutes**. Mix by vortexing briefly several times during incubation.
8	Add 250 µL of ethanol (96-100%) and mix by pipetting or vortexing.
9	Transfer 750 µL of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 30 s at 12,000 × g (~ 11,000 rpm). Discard the flow-through and place the purification column into a new 2 mL collection tube (included). **Note.** Close the bag with GeneJET RNA Purification columns tightly after each use!
10	Proceed to **step 5 of Protocol A**. Total RNA Purification Protocol from 250 µL of fresh whole blood on p. 8.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified RNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols. **The blood cells were not completely lysed.** **Ethanol was not added to the lysate.** Ensure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample briefly by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Ensure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.5. **Inappropriate centrifuge speed.** Please make sure that recommended centrifuge speed was used during purification. It is very important to meticulously adhere to the centrifugation protocol.
Purified RNA is degraded	**RNase contamination.** To avoid RNase contamination wear gloves during the procedure and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Use reagents designed to remove RNase contamination from nondisposable items (pipettes, centrifuges) and work surfaces. **Inappropriate sample storage conditions.** Blood sample stabilized in Lysis Buffer can be stored at 4 °C for no longer than 48 hours. For frozen samples, please make sure that Lysis Buffer was added directly to the frozen blood sample and thawed immediately by vortexing until the sample is thawed. **Purified RNA was not stored properly.** Purified RNA should be used immediately in downstream applications or stored at -20 °C for later use. For prolonged storage (more than 1 month) storage at -70 °C is recommended. **Lysis buffer does not contain DTT.** Ensure that DTT has been added to the Lysis Buffer.
Inhibition of downstream enzymatic reactions	**Purified RNA contains residual ethanol.** If residual solution is observed in the purification column after treating the column with Wash Buffer 2, empty the collection tube and re-spin the column for an additional 1 min at maximum speed (≥ 20,000 × g, ≥ 14,000 rpm). **Purified RNA contains residual salt.** Use the correct order for the Wash Buffers steps. Always wash the purification column with Wash Buffer 1 first and then proceed with Wash Buffer 2.
Column clogging	**Excess starting material was used for lysate preparation.** Reduce the amount of starting material. Do not use more blood or cells than indicated in lysis protocols. **Starting material was not completely lysed.** Reduce the amount of starting material in subsequent preparations.

SAFETY INFORMATION

Lysis Buffer

Xn Harmful
Hazard-determining component of labelling: Guanidinium thiocyanate.

Risk phrases
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed.
R32-52/53: Contact with acids liberates very toxic gas. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases
9 Keep container in a well-ventilated place.
23 Do not breathe gas/fumes/vapour/spray.
36/37/38 Wear suitable protective clothing, gloves, face/eye protection.
60 This material and its container must be disposed of as hazardous waste.
61 Avoid release to the environment. Refer special instructions/ safety data sheets.

Wash Buffer 1

Xn Harmful
Hazard-determining component of labelling: **Guanidinium hydrochloride.**

Risk phrases:
R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

Safety phrases:
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste

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PRODUCT USE LIMITATION

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