Thermo
SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific
GeneJET Whole Blood RNA Purification Mini Kit
#K0761

www.thermoscientific.com/onebio

#K0761
Lot __
Exp. __

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Whole Blood RNA Purification Mini Kit is qualified by isolating total RNA from 500 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 $A_{260/280}$ ratio between 1.9 and 2.1 and the RNA integrity number (RIN) of ≥8.

Quality authorized by: Jurgita Žilinskienė

Rev.3 .

CONTENTS page

COMPONENTS OF THE KIT

GeneJET Whole Blood RNA Purification Mini Kit	#K0761 50 preps
Lysis Buffer	40 mL
Wash Buffer WB 1 (concentrated)	40 mL
Wash Buffer 2 (concentrated)	23 mL
Water, nuclease-free	30 mL
GeneJET RNA Purification Columns pre-assembled with Collection Tubes	50
Collection Tubes, 2 mL	50
Collection Tubes, 1.5 mL	50

STORAGE

All kit components 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 Purification Mini Kit is designed for rapid and efficient purification of high quality total RNA from whole blood and related body fluids. 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 standard procedure takes less than 15 minutes following cell lysis. 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. 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 from various sources.

Source	Amount	Yield, µg
Human blood	500 µL	1.2-1.8
Mouse blood	500 µL	10-11
Rat blood	500 µL	7.4
Rabbit blood	500 µL	10
Bone marrow	350 µL	1.7
Buffy coat	500 µL	2-3

IMPORTANT NOTES

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

	#K0761 50 preps	
	Wash Buffer WB 1	**Wash Buffer 2**
Concentrated wash solution	40 mL	23 mL
Ethanol (96-100%)	**4.5 mL**	**39 mL**
Total volume:	44.5 mL	62 mL

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

- Before each RNA purification experiment prepare a fresh aliquot of Lysis Buffer supplemented with β-mercaptoethanol. Add 20 µL of 14.3 M β-mercaptoethanol to each 1 mL of **Lysis Buffer** used. Store at +4°C for up to 1 month.
- 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.
- Wear gloves when handling the **Lysis Buffer and Wash Buffer WB 1** as these solutions contain irritants (*see* p. 8 for SAFETY INFORMATION) and are harmful if they come into contact with skin, or are inhaled or swallowed.
- Unless otherwise indicated all purification steps are preformed at room temperature (15-25°C).
- Centrifugation speed in rpm's is given for 24-place microcentrifuges.
- Typically the purified RNA has an $A_{260/280}$ 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.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- β-mercaptoethanol
- Pipettes and pipette tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Disposable gloves

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 blood cells should be carried out in the same day**. Samples can be stored at 4°C for no longer than 5 hours. Do not freeze blood samples.
- If it is not possible to process samples the same day, RNA can be preserved in the supplied Lysis Buffer:
  - Centrifuge blood for 5 min 400 × g (~2,000 rpm) at 4°C
  - Discard the supernatant
  - Resuspend the pellet in 600 µL of Lysis Buffer, **mix well**.

Stabilized sample can be stored for 24 h at 4°C, or up to one week at -20°C.

PROTOCOLS

Protocols for RNA purification from buffy coat and bone marrow are described on p.6.

A. Mammalian Whole Blood RNA Purification Protocol

Step	Procedure
1	Centrifuge 50-500 µL of blood for 5 min, 400 × g (~2,000 rpm) at 4°C. Discard the supernatant.
2	Resuspend the pellet in 600 µL of Lysis Buffer, **mix well** by vortexing.
3	Add 450 µL of ethanol (96-100%) and mix by pipetting or vortexing.
4	Transfer half of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through solution and reassemble the column and collection tube. Transfer remaining lysate into the column and centrifuge as before. 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	Add 700 µL of Wash Buffer WB 1 (with ethanol added). 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.
6	Add 500 µL of Wash Buffer 2 (with ethanol added) to the purification column. Centrifuge for 1 min at 12,000 × g (~11,000 rpm).
7	Add 500 µL of Wash Buffer 2 (with ethanol added) to the purification column. Centrifuge for 2 min at 12,000 × g (~11,000 rpm). *Recommended*: Empty the collection tube. Place the purification column back into the tube and re-spin 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.
8	Add 50 µL of nuclease-free water to the centre of the purification column membrane and centrifuge for 1 min at 12,000 × g (~11,000 rpm).
9	Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20°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.

B. RNA Purification from Bone Marrow

Note. Bone marrow that has been previously frozen does not sediment during centrifugation. Use up to 350 µL of thawed bone marrow and proceed directly to step 2 of the standard mammalian Whole Blood RNA Purification Protocol.

Step	Procedure
1	Take 50-500 µL of **fresh** bone marrow.
2	Centrifuge 50-500 µL of bone marrow for 5 min 400 × g (~2,000 rpm) at 4°C. Discard the supernatant.
3	Proceed to step 2 of the mammalian Whole Blood RNA Purification Protocol on p. 5.

C. RNA Purification from Buffy Coat

Buffy coat is a leukocyte-enriched fraction of whole blood and containes approximately 5-10 times more nucleic acids than an equivalent volume of whole blood. Prepare the buffy coat by centrifuging whole blood at 2,000 × g for 10 minutes at room temperature. 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.

Step	Procedure
1	Centrifuge 10 mL of whole blood at 2,000 × g for 10 minutes at room temperature. Three layers should be visible.
2	Remove upper clear layer by aspiration.
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 red blood cell Lysis Solution (10 mM Tris-HCl, pH 7.0, 5 mM $MgCl_2$, 10 mM NaCl) and resuspend WBCs.
5	Centrifuge at 2,000 × g for 10 minutes at room temperature. Remove supernatant. **Do not discard the pellet**.
4	Proceed to step 2 of the mammalian Blood RNA Purification Protocol on p. 5.

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. **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 WB 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.3.
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 cells stabilized in Lysis Buffer can be stored at 4°C for no longer than 24 hours or at -20°C for no longer than 7 days. **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 β-mercaptoethanol .** Ensure that β-mercaptoethanol 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 WB 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 Solution

Xn Harmful

Hazard-determining component of labelling: **Guanidinium thiocyanate.**

Risk phrases

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

Safety phrases

S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.

Wash Buffer WB 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

S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.