RNAbler – Blood

For rapid extraction of total RNA (> 200 bases) from white blood cells using spin-column technology

This product is shipped at room temperature. Upon receipt, store the WBC Pelleting Solution 20X at 4°C and all other components at room temperature.

This product is intended for research use only (RUO).
It is not intended for use in medical diagnosis.

Made in
SAUDI ARABIA
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Table of content

A Kit overview

- Specifications and intended use
- Safety precautions
- Before first use
- Components
- Storage and stability
- Working with RNA

B Protocol for RNA extraction from whole blood

C Appendix

- Preparation of 1X WBC pelleting solution
- Optional DNase I treatment
- Troubleshooting
- Quality control
- Support

Specifications and intended use

This kit is intended for extraction of total RNA from white blood cells. Our direct comparisons show that addition of β-mercaptoethanol is not needed with this kit, which limits exposure to harmful chemicals.
RNA species smaller than 200 bases are not efficiently extracted by this kit. Please refer to our other products that specialize in small RNA extraction.
THIS KIT SHOULD NOT BE USED FOR MEDICAL OR DIAGNOSTIC PURPOSES.

Safety precautions

The Lysis Buffer contains guanidine salts, which are known irritants that are harmful if inhaled, swallowed, or came in contact with skin. Wear appropriate protective equipment and be extremely cautious when handling and discarding the lysis buffer.
Do NOT mix the Lysis Buffer with bleach containing cleaning products.
Refer to the product's webpage for full SDS sheets.

Before first use

It is imperative to add 96-100% ethanol to Wash2 Buffer before first use, as follows:

RE96050 → 48 mL EtOH

Components

RE95050 (50 extractions)	
Lysis Buffer	30 mL
Wash1 Buffer	30 mL
Wash2 Buffer (after ethanol addition)	60 mL
Elution Buffer	5 mL
WBC Pelleting Solution 20X	25
Spin columns*	50

*** nucleic acid binding capacity ~50µg**

Additionally, you will need the following items that are not supplied with the kit:

- 1.5mL DNase/RNase-free tube per sample (elution tube)
- 15mL DNase/RNase-free tube
- Centrifuge with a rotor for 1.5mL tubes capable of producing at least 10,000g
- Centrifuge with a rotor for 15mL tubes
- Pipettes, preferably dedicated for RNA work, and RNase-free tips
- Vortex
- 96-100% ethanol
- Distilled water

Storage and stability

All components are shipped and stored at room temperature. Upon receipt, store the WBC Pelleting Solution 20X at 4°C. Store the rest of components at room temperature. Do not store in humid areas. All components are stable for at least a year from the production date.

Working with RNA

RNA is destroyed by RNases, which are abundant in the air and in human skin. They are very stable enzymes that do not require cofactors and can survive elevated temperature, autoclaving, and cleaning with 70% ethanol. Be sure to use RNase-free plasticware in all steps involved in the handling of RNA. It is also advisable to use dedicated pipettes and space for RNA work, and to change your gloves every time before handling RNA-containing tubes.

Protocol for RNA extraction from whole blood

Our protocols are compatible with freshly drawn blood (with or without EDTA). Do not use blood with added Heparin, as heparin is known to inhibit PCR.

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature.

1. Prepare a sufficient amount of 1X WBC pelleting solution (see appendix)
2. Gently invert the blood tube upside down several times, then transfer 500-1,000µL of whole blood to a new RNase-free 15mL tube
3. Add 5mL of freshly prepared 1X WBC Pelleting Solution, mix by gently inverting the tube upside down 5-10 times, put the tube on ice for 10 minutes with occasional mixing by inversion
4. Centrifuge the tube at 1,000g for 10 minutes, the solution should turn clear red with a white pellet at the bottom with some red discoloration, this pellet contains the white blood cells

   → *If no pellet is visible, repeat centrifugation at 1,000g for 20 minutes*
5. Carefully and slowly pour out the supernatant then add 2.5mL of the 1X WBC Pelleting Solution to the pellet, vortex briefly until the pellet is completely resuspended, then centrifuge at 1,000g for 10 minutes

   → *If the pellet is disturbed or gets resuspended at any point in this protocol, centrifuge the tube at 1,000g for 10 minutes then carry on with the procedure from the point it has stopped*
6. Carefully and slowly pour out the supernatant, use a 100-200µL pipette to remove as much of the supernatant as possible (some red discoloration may still be seen on the pellet, but it should not interfere with the quality of extraction)
7. To the isolated pellet add 500µL of the Lysis Buffer, vortex thoroughly until the pellet is completely resuspended
8. Add 500µL of 96-100% ethanol, vortex thoroughly for 30-60 seconds
9. Apply up to 700µL of the lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube
10. Apply the remaining lysate-ethanol mixture, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column

11. **Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through**

    → *If you opted for the optional DNase I treatment (see appendix), do it here*

12. **Add 500µL of Wash1 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube**

13. **Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through**

14. **Add 500µL of Wash2 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube**

15. **Repeat steps 13-14 for a second washing step with Wash2 Buffer**

16. **Discard the collection tube and place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-100µL of Elution Buffer directly to the center of the spin column filter without touching the filter with the tip, incubate for 2 minutes at room temperature**

    Hint: pre-heating the Elution Buffer at 60-70°C may increase the yield

17. **Centrifuge, discard the spin column, the RNA-containing eluate is ready for use in downstream analysis (use immediately or store in -20°C for short term storage, -80°C for long term storage)**

18. **Measure the purity of your eluate with a spectrophotometer, pure RNA should have 260/280 ratio ~2 and 260/230 ratio ~2.2**

Preparation of 1X WBC pelleting solution

To prepare a sufficient amount of 1X WBC pelleting solution, mix the concentrated 20X solution with distilled water as follows:

Amount of 20X solution = no. of samples X 0.4mL
Amount of distilled water = no. of samples X 7.6mL

- For example, if you have 16 samples:

Amount of 20X solution = 16 X 0.4mL = 6.4mL
Amount of distilled water = 16 X 7.6mL = 121.6mL

Optional DNase treatment

Some sensitive applications may require pure RNA (completely free of DNA), which can be easily obtained with this kit by using an external DNase I set from a trusted manufacturer. Simply follow these guidelines to incorporate an on-column DNase I digestion in your protocol:

- Before starting the experiment, prepare the required number of units of DNase I recommended by the manufacturer in a final volume of 50µL of the DNase I reaction buffer (per sample), leave the tube on ice and start the experiment normally
- When you reach the binding step and centrifuge at 10,000g for 1 minute, discard the flow-through, but do not proceed to the first washing step with Wash1 Buffer; instead, apply the 50µL DNase I working buffer (that you prepared previously and set on ice) directly to the center of the dried spin column
- Incubate at room temperature for the recommended time by the manufacturer (usually 15-30 minutes) then centrifuge at 10,000g for 1 minute
- Discard the flow-through, repeat the first washing step with Wash1 Buffer, and proceed with the protocol from that point

Troubleshooting

Problem	Possible reason	Solution
Spin column clogged	• Too much sample was used	• Up and down pipetting and centrifugation at 15,000g for 3 minutes in the binding step may salvage your sample, but next time use less amount of sample
	• Sample was not homogenized properly	• Ensure sufficient homogenization and lysis
	• Too much cell debris in the lysate	• (For WBCs) centrifuge lysate at 15,000g for 10 minutes, obtain supernatant and discard the pellet
Low RNA yield	• Little or no Ethanol added to lysate	• Make sure to add ethanol before binding
	• Ethanol was not added to Wash2 Buffer before use	• Make sure to add ethanol to Wash2 Buffer as described
	• Too much sample was used	• Use less amount of sample
Degraded RNA	• RNase contamination	• Use RNase-free water and plastic-ware
	• Improper handling or storage of the sample before lysis	• Make sure to use fresh blood

Quality control

All lots are tested by extracting RNA from 1mL whole blood, running it on an RNase-inhibiting agarose gel, and visually inspecting the 28S and 18S bands at around ~5kb and ~1.9kb. To pass the QC test, the 28S band should have almost double the intensity of the 18S without any visible smear on the lane.

Additionally, purity is assessed by spectrophotometry (260/280 and 260/230 ratios).

Support

For questions, suggestions, or technical support, feel free to contact us by email on: **support@havensci.com**

HavenSci

RNAbler – Blood
Product Manual (RE96050)
Version 3.0
www.havensci.com