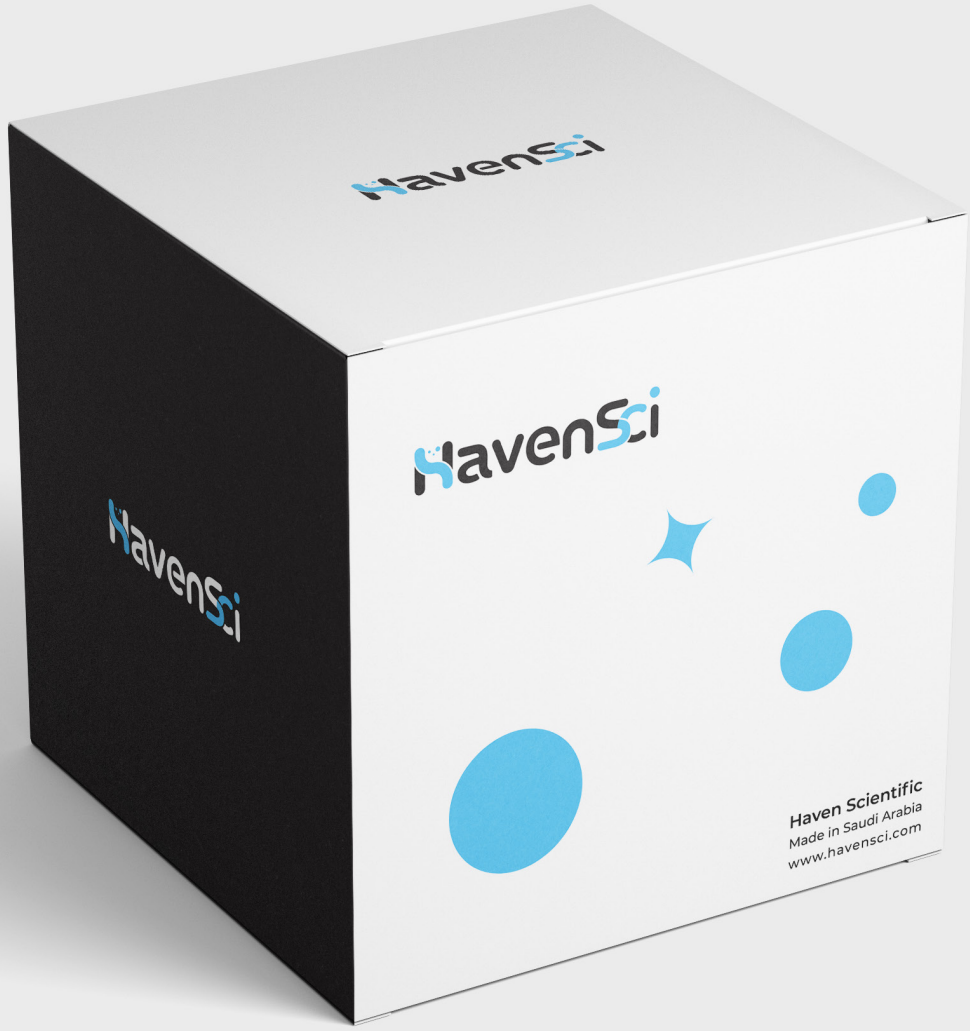
RNAbler – Cells and Tissue

For rapid extraction of total RNA (> 200 bases) from cells and tissueby silica gel adsorption



This product is shipped and stored 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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Specifications and intended use

This kit is intended for rapid extraction of total RNA from mammalian cells and tissue, or to cleanup RNA after DNase treatment or to remove excessive salts. An experienced user can extract up to 6-10 animal cell pellet samples simultaneously in approximately 15 minutes (tissue samples may take a few minutes longer).

Our direct comparisons show that addition of β-mercaptoethanol is not needed in this kit, which limits exposure to harmful chemicals.

RNA species smaller than 200 bases are not efficiently extracted with 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 hydrochloride, which is a known irritant that is 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 Lysis/Binding Buffer with bleach. 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:

RE95050 → 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
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)
- Centrifuge with rotor for 1.5mL tubes capable of producing at least 10,000g
- Pipettes, preferably dedicated for RNA work, and RNase-free tips
- 96-100% ethanol
- For tissue samples: rotor-stator homogenizer OR bead-beater homogenizer OR mortar and pestle with liquid nitrogen

Storage and stability

All components are shipped and stored at room temperature. Do not store in humid areas. All components are stable for at least a year from the indicated 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 plastic ware in all steps involving 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.

Mammalian tissue

Use this protocol to extract RNA from up to 25mg (10mg of spleen) of fresh tissue or tissue preserved in RNALater (or equivalent). For tissue smaller 5mg use 300µL Lysis Buffer. For tissue 5-25mg use 500µL of Lysis Buffer. For tissue preserved in RNALater, simply dab the tissue on lint-free paper towel before mixing it with the Lysis Buffer.
Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature.

1. Lyse and homogenize up to 25mg of tissue by one the following methods:
   - a. Cut the tissue to small pieces, add it to the required amount of Lysis Buffer and homogenize with a rotor stator homogenizer following the instrument's instructions; 15-30 seconds should be sufficient for complete lysis
   - b. Add the required amount of Lysis Buffer to a 2mL tube, cut the tissue to small pieces and add it to the tube, add 1-2mm sterile steel beads, homogenize with a bead beater following the manufacturer's instructions
   - c. Place the tissue on a mortar, add liquid nitrogen, crush it thoroughly with a pestle into a fine powder, allow the liquid nitrogen to evaporate, quickly transfer the powder to a tube containing the required amount of Lysis Buffer, vortex briefly
   - d. (optional for soft tissue, e.g. brain, kidney, etc. <5mg) cut the tissue to small pieces, add it to the required amount of Lysis Buffer and homogenize with a manual sterile pestle that fits the bottom of your tube until no small pieces are visible

   → *If the lysate contains excess cell debris, pellet the debris by centrifugation at >14,000g for 5 minutes, then carefully transfer as much as possible of the supernatant to a clean DNase/RNase-free 1.5mL tube*

   → *Stopping point: you may store the lysate in -20°C for up to a year at this point*

2. Add a volume of 96-100% ethanol equal to that of the lysate (e.g. if you used 500µL Lysis Buffer, add 500µL ethanol), vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)
3. 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

4. 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
5. 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*
6. 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
7. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through
8. 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
9. Repeat steps 7-8 for a second washing step with Wash2 Buffer
10. 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
11. 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)
12. Measure the purity of your eluate with a spectrophotometer, pure RNA should have 260/280 ratio ~2 and 260/230 ratio ~2.2

Mammalian cells

With this protocol RNA can be extract from 10^4-10^7 mammalian cells.
Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature.

1. To digest cells, use step 2a for adherent cells and step 2b for suspension cells:
   a. Discard as much media as possible, rinse with PBS, gently scrape the cells and transfer them to a clean DNase/RNase-free 1.5mL tube
   b. Pellet the cells by centrifugation at 1,000g for 10 minutes, discard the cell media (be careful not to disrupt the pellet), use a 10-20µL pipette to remove the remaining media from around the pellet
2. Add 300µL of Lysis Buffer to the cells (if starting number of cells >10^6, use 500µL of Lysis Buffer), vortex briefly and spin down

   Hint: if the starting number of cells is too low, incubating the lysate at 60°C for 10 minutes with occasional vortexing may improve the yield

   → *Stopping point: you may store the lysate in -20°C for up to a year at this point*
3. Add a volume of 96-100% ethanol equal to that of the lysate (e.g. if you used 500µL Lysis Buffer, add 500µL ethanol), vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)
4. 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
5. 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
6. 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*
7. 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

8. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through
9. 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
10. Repeat steps 8-9 for a second washing step with Wash2 Buffer
11. 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: if the starting number of cells is too low, incubating the lysate at 60°C for 10 minutes with occasional vortexing may improve the yield
12. 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)
13. Measure the purity of your eluate with a spectrophotometer, pure RNA should have 260/280 ratio ~2 and 260/230 ratio ~2.2

RNA Cleanup

This is a quick, phenol-free protocol to cleanup your RNA samples after off-column DNase treatment, or to remove impurities.
Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature.

1. Adjust the volume of your RNA sample to 100µL with Elution Buffer or RNase-free water
2. Add 300µL of Lysis Buffer, followed by 400µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)
3. Apply the whole 800µL of the RNA-Lysis Buffer-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
4. 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*
5. 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
6. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through
7. Add 500µL of Wash2 Buffer to the spin column, centrifuge at 12,000g for 2 minutes, discard the collection tube and proceed to the next step
8. 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 at room temperature for 2 minutes

   Hint: pre-heating the Elution Buffer at 60-70°C may improve the yield
9. Centrifuge, discard the spin column, the RNA-containing eluate is ready for use in downstream analysis (use immediately or place at -20°C for short term storage, -80°C for long term storage)
10. Measure the purity of your eluate with a spectrophotometer, pure RNA should have a 260/280 ratio ~2 and a 260/230 ratio ~2.2

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, and proceed to the first washing step

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 debris in the lysate	• Centrifuge lysate at 15,000g for 3 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 put the sample in -80°C or in RNALater immediately after acquisition

Quality control

All lots are tested by extracting RNA from a pellet of human blood cells, 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 – Cells and Tissue
Product Manual (RE95050)
Version 3.0
www.havensci.com