Viral RNA Extraction Kit

For RNA extraction from clinical samples and biological fluids using spin-column technology.

Catalog Numbers:

RE97010

RE97050

RE97250

Product Manual (Version 1.0)

This product is shipped in two boxes. Box1 components should be stored at room temperature. Box2 should be placed at -20°C immediately upon receipt.

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

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A. **Kit overview**

- **Specifications and intended use**

This kit is intended for RNA extraction from clinical samples. The procedure is quick and simple, and is suitable for low- to mid- throughput applications. An experienced user can process up to 16 samples in less than an hour.

Our protocols are compatible with viral transport media (VTMs), plasma, serum, cerebrospinal fluid (CSF), saliva, and whole blood. Do not use blood supplemented with Heparin, as heparin is known to inhibit PCR.

Although the kit is intended for viral RNA extraction, please note that all RNA species in the samples are extracted simultaneously. For example, if the sample is derived from a human origin, human RNA is extracted along with viral RNA (if any).

THIS KIT SHOULD **NOT** BE USED FOR MEDICAL OR DIAGNOSTIC PURPOSES.

- **Safety precautions**

The Lysis Buffer contains a guanidine salt, 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 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:

RE97010 → 8 mL EtOH

RE97050 → 48 mL EtOH

RE97250 → 240 mL EtOH

- **Components**

		RE97010 (10 extractions)	RE97050 (50 extractions)	RE97250 (250 extractions)
Box 1	**Lysis Buffer**	10 mL	30 mL	150 mL
	Wash1 Buffer	10 mL	30 mL	150 mL
	Wash2 Buffer (after ethanol addition)	20 mL	60 mL	300 mL
	Elution Buffer	2 mL	5 mL	25 mL
	Spin columns*	10	50	250
Box 2	**Proteinase K**	0.2mL	1mL	5mL

* 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
- Vortex
- Pipettes and DNase/RNase-free tips
- 96-100% ethanol

- **Storage and stability**

This kit is shipped in two boxes. Store Box2 at -20°C immediately upon receipt. Store all other components of Box1 in a dry area at room temperature. All components of the kit are stable for at least a year from the indicated production date.

B. **Protocol for viral RNA extraction**

This protocol is compatible with a wide range of clinical samples including viral transport media (VTMs), serum, plasma, whole blood, cerebrospinal fluids (CSF), and saliva. If you are using a different type of samples, please contact our technical support team.

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

1. Switch on a water bath or a heat block and set it on 55-60°C

2. Transfer 200µL of the sample to a separate clean, sterile 1.5mL tube

3. Add 20µL of proteinase K and 200µL of Lysis Buffer, vortex briefly and spin down, then incubate at 55-60°C for 10 minutes with occasional vortexing (for saliva, increase incubation to 30-60 minutes, or until the sample is completely homogenized)

   ➔ ***A fresh master mix of Lysis Buffer/proteinase K may be prepared***

4. Spin down the tube to collect droplets that have condensed on the inside of the lid then add 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)

5. Apply the whole 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

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

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 10 seconds then proceed to the next step without discarding the flow-through

9. 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

10. Place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-50μL 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 increase the yield

11. Centrifuge, discard the spin column, the 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)

12. RNA concentration in many clinical samples (e.g. serum, plasma, VTM, etc.) is usually below the detection limit of most spectrophotometric techniques. We recommend you to assess the RNA directly by using it in the downstream application (PCR, qPCR, library preparation, etc.)

C. **Appendix**

- **Troubleshooting**

Problem	**Possible reason**	**Solution**
Spin column clogged	▪ Too much sample was used	▪ Pipetting the lysate in the clogged column up and down followed by another centrifugation at 15,000g for 3 minutes in the binding step may salvage your sample, but next time use less amount of sample
	▪ Proteinase K was not added	▪ Ensure addition of proteinase K as recommended in the protocol
	▪ Too much debris in the lysate	▪ Centrifuge lysate at 15,000g for 3 minutes, obtain supernatant and discard the pellet
Low 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

- **Quality control**

All lots are tested by for predetermined set of parameters to ensure consistent performance among all lots produced.

- **Support**

For questions, suggestions, or technical support, feel free to contact us by email on:

support@havensci.com