SHENTEK

Residual Host Cell RNA Sample Preparation Kit User Guide

Version: A/2
For Research Use Only
Product No.: 1201205
Reagents for 100 Extractions

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

SHENTEK® Residual Host Cell RNA Sample Preparation Kit utilizes magnetic particle-based separation technique for RNA extraction from biological products. Extracted RNA can be used in various downstream RNA qauantitation applications (*E. coli*, 293T etc.). This kit is compatible with automated extraction using our rHCDpurify instrument (recommended) as well as a sophisticated manual sample preparation procedure.

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions.Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit components and storage

No.	Reagent	Part No.	Quantity	Storage
I	Proteinase K Buffer	NND026	10 mL × 1 bottle	room temperature
	Binding solution	NND016	20 mL × 1 bottle	
	Wash buffer A	NND014	30 mL × 1 bottle	
	Elution solution	NND061	10 mL × 1 bottle	
	Dilution solution	NND062	12 mL × 2 bottles	
II	Magnetic particles	NND030	750 μL × 2 tubes	2-8℃
	5M NaCl	NND040	500 μL × 2 tubes	
III	Proteinase K	NND023	500 μL × 2 tubes	-20℃
	RNase inhibitor	NND060	50 μL × 1 tube	
	Glycogen	NND035	500 μL × 2 tubes	
	Precipitation solution I	NND003	25 μL × 1 tube	

The kit components can be stored at the appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Required materials not included in the kit

- Anhydrous Ethanol (Analytical Reagent, AR)
- 100% Isopropanol (AR)
- 1 M HCl or 1 M NaOH (optional)
- Low retention filter tips: 1000 μL, 100 μL, 10 μL
- Nonstick, RNase-free microcentrifuge tubes 1.5 mL

■ Related equipment

- Benchtop microcentrifuge
- Magnetic Stand or rHCD purify instrument
- Vortex mixer
- Dry bath incubator
- Micropipettes, 1000 μL, 100 μL, 10 μL
- Real-time PCR System
- Biosafety cabinet

2. Methods

■ Experiment preparation

Before first use of the kit:

- Add 40 mL of ethanol to Wash buffer A (NND014).
- Prepare a 70% ethanol buffer with Dilution solution (NND062) in a clean tube, label as Wash buffer B.
- Store Wash buffer A & B at room temperature properly to prevent evaporation by expiration date.

Before each use of the kit:

- Prepare 100% isopropanol.
- Set the dry bath temperatures to 55°C, 50°C or 37°C.

Note: If the reagent is cloudy or contains precipitates, heat at 37°C until it clears.

➢Proteinase K digestion solution preparation, please refer to Table 2.

Table 2. Proteinase K digestion solution preparation

Protein concentration (mg/mL) in the sample	Proteinase K (NND023) volume (μL/sample)	Proteinase K Buffer (NND026) volume (μL/sample)
0-100	10	100
100-200	20	

Note:

(1) Ignore this step if the test sample does not contain any protein (such as plasmid DNA).

(2) Prepare the total volume of Proteinase K digestion solution appropriately more than necessary for experiment to compensate for pipetting loss.

(3) If Proteinase K Buffer is cloudy or contains precipitates, heat at 37°C until it clears, and mix well.

(4)The effect of Proteinase K digestion may influence RNA recovery and detection.

➢ Preparation of working elution solution

In the negative operation zone, prepare the working elution solution following the ratio of RNase inhibitor (NND060): Elution solution (NND061) = 1 : 200 (v:v).

➢ Preparation of working binding solution

a. Precipitation solution I dilution: According to the volume of binding buffer needed for the experiment, prepare the volume of Precipitation solution I and dilute it with Dilution solution (NND062) at a volume ratio of 1:99.

b. working binding solution preparation (for one sample): Add 200 μL of Binding solution (NND016), 10 μL of diluted Precipitation solution I (please see Binding buffer preparation - Step a) and 10 μL of Glycogen (NND035).

■ Samples preparation

- Sample dilution

  Test samples may contain high levels of RNA that are above the upper limit of quantification for the residual RNA assay. Please dilute the samples with Dilution solution (NND062) before RNA extraction.

  Note: You may also dilute samples with a post-extraction working elution solution.

- Sample reconstitution

  If the sample is a dry powder, it needs to be dissolved with the Dilution solution (NND062) or other buffer to reach a high-concentration sample solution, further dilution can be performed with the Dilution solution (NND062) before the subsequent steps.

- pH of test samples

  The ideal sample pH is neutral, if the sample pH < 5.0 or pH> 9.0, it may affect the purity of extracted RNA. Therefore, test the sample pH and adjust the pH to neutral (pH 6.0-8.0) with 1 M HCl or 1 M NaOH before sample preparation.

- Negative Control Sample (NCS)

  The NCS is necessary in this assay that are treated along the entire flow with unknown test sample. The NCS serves as a blank in each experiment to evaluate whether there is cross contamination or environmental contamination during sample handling.

  Note: If the sample is diluted, please use the same buffer for negative controls.

- Sample Extraction Recovery Control (ERC)

  Sample extraction recovery control (ERC) is used to evaluate the efficiency, recovery rate and accuracy of RNA extraction, the performance of assay validation and systematic compatibility. The amount of ERC added to the sample is recommended to be 2 to 10 times of the sample detection value.

- Plasmid DNA samples amplified from *E. coli* host bacteria

  For the preparation of NCS, Samples, ERC, please refer to SHENTEK® Residual *E.coli* RNA Quantitation Kit (2G) User Guide (Product No. 1201201-1) for DNase I digestion.

■ Sample digestion

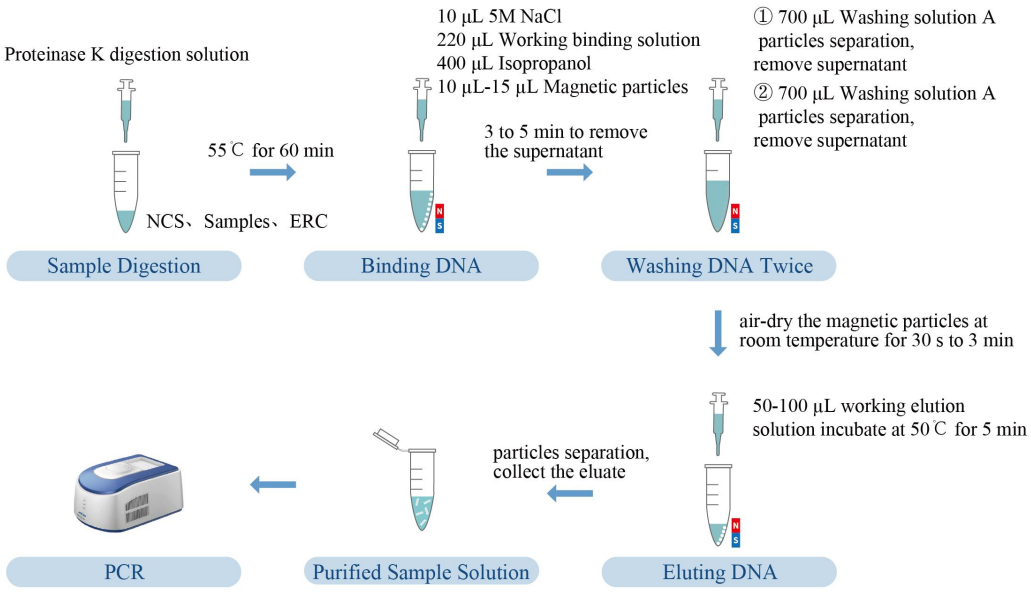
Note: If it is known that the test sample does not contain protein (such as plasmid DNA), please ignore this step.

For each sample tube:

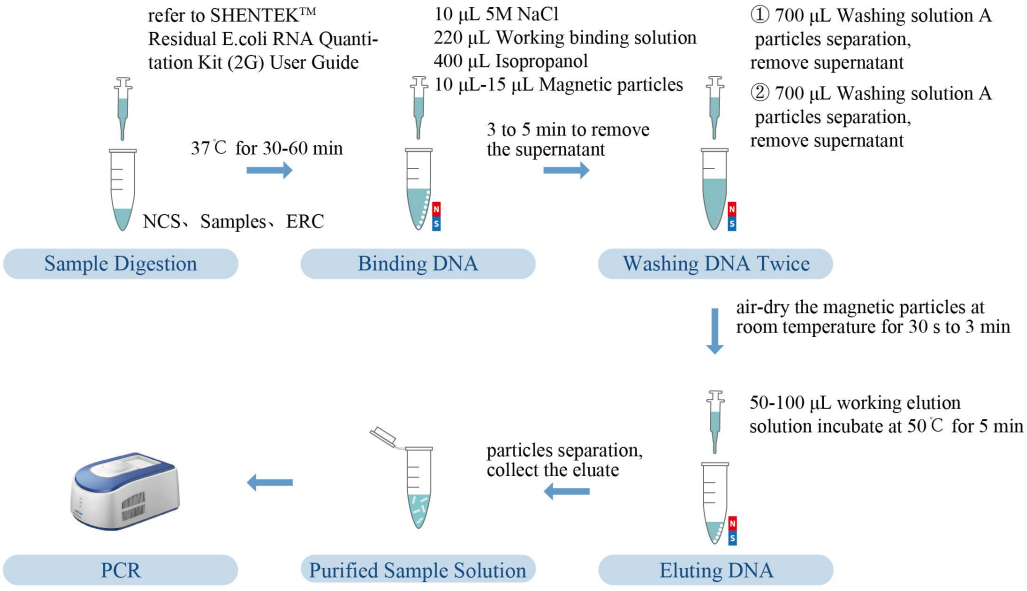
1. Add 100 µL sample and 110 µL or other volumes Proteinase K digestion solution, then vortex and centrifuge briefly in a microcentrifuge.
2. Incubate at 55℃ for 60 min in dry bath incubator.
3. After this step, machine extraction or manual extraction can be selected following individual step blow. For machine extraction procedure, a maximum digestion volume of 300 µL can be managed in rHCDpurify system.

■ RNA extraction (Manual)

1. The sample containing protein



2. Plasmid DNA samples amplified from *E. coli* host bacteria



Note: After sample preparation, the following RNA extraction experiment should be processed as soon as possible.

Binding RNA

For each sample tube:

1. Add 10 μL 5M NaCl (NND040) and 220 μL working binding solution, vortex and brief spin for 10 seconds in a microcentrifuge.
2. Add 200 μL to 400 μL isopropanol, vortex and spin for 10 seconds.

Note:The volume of isopropanol is approximately 1/3 to 1/2 of the total liquid volume.

3. Add 10 μL-15 μL Magnetic particles (NND030), vortex the tubes vertically at medium speed for 5 minutes to bind the nucleic acids. Spin for 10 s and place the tubes on the magnetic stand.

Note:

(1) Before use, vortex the Magnetic particles for 5 seconds until the particles are completely re-suspended Magnetic particles must be re-suspended before adding to each sample to ensure the consistency.

(2) Centrifugation is needed to spin the particles and eluent from

the cap to the bottom of the tube.

4. Wait until the solution is clear and the *particles* are completely separated, discard the supernatant without disturbing the *particles*.

Note:

(1) Normally, the particles separation takes 3-5 minutes.

(2) Do not disturb the Magnetic particles when aspirating the supernatant. During extraction, always place the magnetic bead pellet toward the magnet stand.

Washing RNA

For each tube of Magnetic particles (bind with nucleic acids):

1. Add 700 μL of Wash buffer A, vortex for 10 s to mix well, then spin for 10 s and place the tubes on the magnetic stand.Wait until the solution is clear and the particles are completely separated. Discard the supernatant without disturbing the Magnetic particles.

2. Add 700 μL of wash buffer B, vortex for 40 s to mix well, then spin for 10 s and place the tubes in the magnetic stand.Wait until the solution is clear and the particles are completely separated. Discard the supernatant without disturbing the Magnetic particles.

3. To remove the supernatant completely, spin for 10 s in a microcentrifuge and place the tubes in the magnetic stand. Wait until the particles are completely separated, carefully use a 10 μL volume pipette to remove the remaining liquid.

Note: When removing the supernatant, avoid removing the Magnetic particles together with the supernatant.

4. With the cap open, air-dry the magnetic particles at room temperature for 30 seconds to 3 min to remove any residual ethanol.

Note: The drying time depends on the environmental conditions. It could be shorter in higher temperature or low humidity condition, while slightly longer in lower temperature or high humidity condition.

Elution RNA

For each sample:

1. Add 50-100 μL working elution solution, vortex for 5 seconds

and incubate in the dry bath at 50℃ for 5 min. Vortex 2–3 times during incubation to ensure complete resuspension of the Magnetic particles.

Note:

(1) Vortex the mixture of Magnetic particles and eluent, then swing to the bottom of the tube.

(2) If the mixture splashed to the cap, centrifuge the tube and mix well by vortex.

2. After incubation, Quickly spin the eluate tube for 1 min , then place the tubes on the magnetic stand. Wait until the particles are completely separated, carefully transfer the eluate to a clean microcentrifuge tube.

3. Quickly spin the eluate tube for 10 s and then place it on the magnetic stand. Wait until the particles are completely separated, carefully transfer the eluate to a nonstick 1.5 mL microcentrifuge tube and label the corresponding sample.

Note: Transfer the eluate completely and avoid leaving any residuals behind.

Precautions

1. During washing and eluting RNA, centrifugation should be performed immediately after vortex to ensure no magnetic particles or liquid left on the tube caps or walls.

2. When open the centrifuge tube, hold the tube in one hand, gently open the cap with your thumb without splashing.

3. Place the tubes in the magnetic stand with the pellet against the magnet, and rotate the tubes slowly during the process to accelerate the Magnetic particles aggregation.

4. Do not over dry the Magnetic particles when removing the residual ethanol, over drying will may influence the elution step.

5. Please perform the subsequent assay on the same day after nucleic acid extraction to ensure the accurate results.

■ Machine extraction（Using rHCDpurify system）

During digestion, add the corresponding solution according to the table 3:

Table 3. 96 deep well plate layout

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
S1											
S2						S1-ERC					
S3						S2-ERC					
S4						S3-ERC					
S5						S4-ERC					
S6						S5-ERC					
						S6-ERC					
NCS											

- Column 1 or 7: working binding solution 220 μL/well, 10 μL 5M NaCl, isopropanol 200 μL to 400 μL/well and all samples after digestion
- Column 2 or 8: Washing buffer A 700 μL/well
- Column 3 or 9: Washing buffer B 700 μL/well
- Column 4 or 10: Magnetic particles 15 μL/well
- Column 5 or 11: working elution solution 100 μL/well

Note:

The volume of isopropanol is approximately 1/3 to 1/2 of the total liquid volume.

Program setting

a. Power button on → click "login" to enter account and password → enter the main page.

b. Wipe the interior of the instrument with a 75% ethanol→click on "UV lamp"→ select "15 minutes".

Note: This step can be set before the extraction preparation operation.

c. Place the sample 96 deep well plate in a fixed position in the instrument and insert the plastic sleeve into the corresponding position of the magnetic head.

d. Click "Run" → select "rHCR-1201205" program → scan the two-dimensional code on the reagent kit→instrument working

e. At the end of the program, a "drip" sound is emitted. Immediately remove the deep well plate and transfer all the purified sample solution to the corresponding new 1.5 mL tube.

Important points to note

a. Before starting the program, it is essential to add plastic sleeve.

b. UV sterilization is required for at least 15 minutes before and after instrument operation. The interval of two extractions will need more than 30 minutes.

c. After the program is completed, the sample eluent needs to be transferred immediately to a clean 1.5 mL tube.

d. Please try to conduct subsequent testing on the same day of sample RNA extraction to ensure an accurate test result.

■ Troubleshooting

Problem	Possible cause	Suggested Solution
Low recovery of nucleic acids	Ethanol not added in Wash buffer A	Add ethanol to Wash buffer A according to the user manual
	Magnetic pellet over drying	The drying time depends on the lab environment. Shorten or extend the incubation time according to above description.
	Magnetic particles are attached too tightly to the tube walls during the elution.	Vortex the microcentrifuge tube with the eluate, until the Magnetic particles fall off the tube walls and suspended in the eluate; if the Magnetic particles are still attached to the tube walls, incubate the microcentrifuge tube at 50°C for 2 min, then vortex until the Magnetic particles are suspended in the eluate.
	Low ion concentration in the sample	Adjust the ionic concentration with 5M NaCl.
	Low pH of sample	Adjust the pH of the samples to neutral.
	High protein content in the sample	Increase Proteinase K volume and digestion time appropriately.
	Loss of Magnetic particles during washing	If the Magnetic particles sink to the bottom of the tube in the magnetic stand, resuspend the Magnetic particles by pipetting gently at the tube bottom until the particles are attached to the walls.
Unstable recovery rate	Store Magnetic particles -20℃ caused the performance drop	Store the Magnetic particles at 2-8℃.
	Inaccurate spiking or aspiration	Use low retention filter tips and calibrate the pipette regularly to guarantee precise measurement.
	Residual Magnetic particles left in the sample after elution	Centrifuge again and place it in the magnetic stand, then carefully transfer the eluate to a clean 1.5 mL tube.

■ References

- USP<1126> Nucleic Acid-Based Techniques — Extraction, Detection, and Sequencing
- EP<2.6.21> Nucleic acid amplification techniques

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Support & Contact

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