SHENTEK

Residual Host Cell DNA Sample Preparation Kit User Guide

Version: A/1
For Research Use Only
Product No.: SK030203D100
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 DNA Sample Preparation Kit uses magnetic particle separation technology to extract residual DNA from a wide range of biological products from various manufacturing steps and types. This kit is suitable for the efficient and reproducible recovery of trace DNA in multiple complex sample matrix. It can be used with SHENTEK® host cell (CHO, *E.coli*, Vero, yeast, NS0, Human, MDCK, Sf9 & AcNPV, Hi5 & AcNPV, plasmid, SV40LTA & EIA et al.) DNA quantitation and size analysis kits.

This kit is compatible with manual sample preparation or automatic extraction using the rHCDpurify instrument.

■ Kit contents and storage

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

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
Wash buffer A	NND014	30 mL × 1 bottle	room temperature
Binding solution	NND016	20 mL × 1 bottle	
Elution buffer	NND018	10 mL × 1 bottle	
Dilution buffer	NND021	10 mL × 1 bottle	
Proteinase K Buffer	NND026	10 mL × 1 bottle	
Magnetic particles	NND031	1 mL × 1 tube	2-8℃
Proteinase K	NND023	500 μL × 2 tubes	-20℃
Glycogen	NND035	500 μL × 2 tubes	
Yeast tRNA	NND037	50 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 (AR)
- 100% Isopropanol (AR)
- Nonstick, Low Retention Tips, 1000 µL, 100 µL, 10 µL
- Nonstick, RNase-free Microfuge Tubes (1.5 mL or 2 mL, 50 mL)
- 1M HCl & 1M NaOH
- 5M NaCl
- PBS (1X, pH 7.4, free of Mg and Ca)
- PCR 8-well strip tubes with caps or 96-well plates with seals
- Nonstick, RNase-free microcentrifuge tubes 1.5 mL
- Low retention filter tips 1000 µL, 100 µL, 10 µL

■ Related equipment

- Benchtop microcentrifuge
- Vortex mixer
- Dry bath incubator
- Magnetic stand or rHCDpurify
- Pipettes,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 Anhydrous Ethanol to Wash buffer A (NND014).
- Prepare a 70% Anhydrous Ethanol buffer with ddH_2O in a clean tube, label it with 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 37°C and 55°C.

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

- Proteinase K digestion solution preparation:

1. Proteinase K digestion solution preparation per 100 μL of sample: if the protein concentration is 0-100 mg/mL in the sample, add 10 μL of Proteinase K to 100 μL of Proteinase K Buffer and mix well; if the protein concentration is 100-200 mg/mL in the sample, add 20 μL of Proteinase K to 100 μL of Proteinase K Buffer and mix well.

The appropriate Proteinase K volume for one sample depends on the protein concentration, please refer to Table 2:

Table 2. Proteinase K volume for one sample

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

2. Calculate the total volume of Proteinase K digestion solution required for the experiment based on the amount of Proteinase K and proteinase K buffer needed per sample, the volume and number of samples.

Note: Prepare the total volume of Proteinase K digestion buffer appropriately more than necessary for experiment to compensate for pipetting loss. If Proteinase K buffer (NND026) is cloudy or contains precipitates, heat at 37°C until it clears, and mix well. The effect of Proteinase K digestion influence DNA recovery and detection.

- Binding buffer preparation (for one sample)**:**

200μL Binding solution (NND016) + 9μL Glycogen (NND035) + 0.2μL Yeast tRNA (NND037)

Calculate the volume of Binding solution required for the experiment based on the amount of binding solution volume needed per sample and number of samples.

Note: Do not add Yeast tRNAs when extracting for E.coli and yeast DNAs.

If the reagents are cloudy or precipitate, heat at 37°C until they clear, and mix well.

■ Samples preparation

- Sample dilution

Test samples from the early upstream process may contain high levels of DNA that are above the upper limit of quantitation for the residual DNA assay. Please dilute the samples with PBS (1X, pH 7.4, free of Mg and Ca) or Dilution buffer (NND021) before DNA extraction. You may also dilute samples with a post-extraction DNA dilution buffer. If the sample is diluted, please use the same buffer for negative controls.

- Sample dissolution

If the sample is a dry powder, it needs to be dissolved with the Dilution buffer (NND021) or other buffer to prepare a high-concentration sample solution, then diluted with Dilution buffer (NND021) before the subsequent steps. Generally the dry powder sample is prepared with a final concentration of 10 or 100 mg/mL.

- pH requirement

If the pH of the sample is < 5 or > 9, as with most samples being processed downstream, it will affect the sample preparation. Therefore, test the pH of the sample and adjust the pH to neutral (pH 6.0-8.0) with 1M HCl or NaOH before sample preparation.

- Parallel sample preparation

To ensure the accuracy, it is recommended that each sample is subjected to three preparations and assays in parallel.

- Negative control sample (NCS)

Each experiment requires the NCS as blank sample prepared in the same procedure as unknown test samples to evaluate whether there is cross contamination or environmental contamination during sample handling.

- Sample extraction recovery control (ERC)

Sample extraction recovery control (ERC) is used to evaluate the recovery and accuracy of DNA extraction, the performance of assay validation and system condition. The amount added to the sample is recommended to be 2 to 10 times the amount quantified in the unspiked sample.

■ DNA extraction

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

- Sample digestion

For each sample tube:

1. Add 100 μL sample and 10 μL 5M NaCl, then quickly vortex to mix well.
2. Add Proteinase K digestion solution, vortex to mix well, and incubate at 55℃ for 60 min.

- Binding DNA

For each sample tube:

1. Add the Binding buffer (The Binding buffer should not contain Yeast tRNA for E.coli and Yeast DNA samples), vortex to mix well.

2. Quick spin for 10 seconds in a microcentrifuge, then add 200μL Isopropanol and 10 μL Magnetic particles.

Note: Vortex the magnetic particles for 5 seconds until the particles are completely suspended before use. Magnetic particles must be re-suspended before being added to each sample to ensure consistency in the amount of magnetic particles added to each sample.

3. Vortex the tubes vertically at medium speed for 5 minutes to bind the nucleic acids. Spin for 10 seconds in a microcentrifuge and place the tubes in the magnetic stand.

Note: Centrifuge is needed to spin the particles and eluent from the cap to the bottom of the tube.

4. Until the solution is clear and the particles are completely separated, aspirate the supernatant without disturbing the particles, and discard the supernatant.

Note: The time for complete clear solution and particle separation is about 3-5 minutes.

During the removal of the supernatant, avoid removing the magnetic particles with the supernatant. During extraction, when the sample tubes are placed into the magnetic stand, always orient the Magnetic particle pellet toward the magnet.

- Washing DNA

For each tube with magnetic particles (binding nucleic acids):

1. Add 700 μL of Wash buffer A, vortex for 10 seconds to mix well and spin for 10 seconds in a microcentrifuge, then place the tubes in the magnetic stand. Aspirate the supernatant without disturbing the magnetic particles, then discard the supernatant.

2. Add 700 μL of Wash buffer B, vortex for 40 seconds and spin for 10 seconds, then place the tubes in the magnetic stand. Aspirate the supernatant without disturbing the magnetic particles, then discard the supernatant.

3. To remove supernatant completely, spin for 10 seconds in a microcentrifuge and place the tubes in the magnetic stand. Wait until the particles completely

separate, use a 10 μL volume pipette to carefully 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 pellet at room temperature for 30 seconds to 3 min to remove any residual ethanol.

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

- Eluting DNA

For each sample:

1. Add 50-100 μL pre-warmed (70°C) Elution buffer, vortex for 5 seconds and incubate at 70℃ for 7 min. Vortex 2–3 times during incubation to ensure complete resuspension of the Magnetic particles.

Note: Vortex to shake the Magnetic particles and eluent to the bottom of the tube. If centrifuge is needed to spin the particles and eluent from the cap to the bottom of the tube, vortex it again to mix well.

2. After incubation, centrifuge for 1 min, and then place the tubes in the magnetic stand. Wait until the particles completely separate, carefully transfer the eluate to a clean centrifuge tube.

3. Quickly spin the tube for 10 seconds and then place it in the magnetic stand again. Carefully transfer the eluate to a nonstick 1.5 mL centrifuge tube and label the corresponding sample name.

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

- Precautions

1. During washing and eluting DNA, centrifugation should be performed right after vortex to ensure that no Magnetic particles or liquid on the tube caps or walls.

2. Every time while holding the EP tube in the left 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 particle aggregation.

4. Do not over dry the magnetic particles when removing the residual ethanol; over drying will make the pellets difficult to resuspend in the Elution buffer in next step.

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

■ Troubleshooting

Problem	Possible cause	Suggested Solution
Low recovery of nucleic acids	No ethanol in Wash buffer A	Add ethanol to Wash buffer A according to the procedure
	Magnetic pellet over drying	The drying time depends on the specific environment. It could be 30 seconds to 1 min in higher temperature or lower humidity condition, while 1 to 3 min in lower temperature or higher humidity condition.
	Magnetic particles are attached too tightly to the tube walls during the elution.	Vortex the centrifuge 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 centrifuge tube at 70°C for 2 min, then vortex it until the magnetic particles are suspended in the eluate.
	Poor binding capability of magnetic particles	Pre-aliquot the magnetic particles and repeat incubation at 37°C no more than 5 times.
	Low ion concentration in the sample	Adjust the ionic concentration with 5M NaCl.
	Low pH of sample	Adjust the pH of sample to neutral.
	High protein content in the sample	Increase Proteinase K volume and sample 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 at the bottom by pipetting gently until the particles are attached to the walls.
Unstable efficiency	Below -18℃ storage of magnetic particles caused the performance degradation	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.

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Support & Contact

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