SHENTEK

Residual Host Cell DNA Sample Preparation Kit For Vaccines User Guide

Version: A/0
For Research Use Only
Product No.: SK030206DM50
Reagents for 50 Extractions

Huzhou Shenke Biotechnology Co., Ltd

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

1. Product information

■ Product description

SHENTEK® Residual Host Cell DNA Sample Preparation Kit For Vaccines is based on magnetic particle separation technology, and specifically optimized for vaccine products. The kit is suitable for sample pretreatment of residual DNA in finished vaccines, such as Vero cell-derived rabies vaccines. The kit is able to obtain trace amounts of DNA in samples stably and efficiently, and works together with SHENTEK® host cell DNA qPCR detection kits to provide an integrated workflow from sample preparation to detection assay.

The kit is compatible with either manual sample preparation, or automated extraction using rHCDpurify® system.

If the sample contains aluminum adjuvants or dextran, specific sample processing methods can be provided by Huzhou Shenke Biotechnology Co., Ltd.

■ 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

No.	Reagent	Part No.	Quantity	Storage
I	Wash buffer A	NND015	15 mL × 1 bottle	room temperature
	Binding solution	NND017	10 mL × 1 bottle	
	Elution buffer	NND019	5 mL × 1 bottle	
	Dilution buffer	NND022	5 mL × 1 bottle	
	Sample buffer	NND029	1 mL × 2 tubes	
II	Magnetic particles	NND030	750 μL × 1 tube	2-8℃
III	Proteinase K	NND023	500 μL × 2 tubes	-20℃

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

- Ultra-pure water
- Anhydrous Ethanol (AR)
- 100% Isopropanol (AR)
- 5M NaCl
- 1M HCl & 1M NaOH
- Low retention, sterile filter tips: 1000 µL, 100 µL, 10 µL
- Low retention, RNase/DNase-free, sterile microcentrifuge tubes, 1.5 mL
- PCR 8-well strip tubes with caps or 96-well plates with seals

■ Related equipment

- Benchtop microcentrifuge
- Vortex mixer
- Dry bath incubator
- Magnetic stand or rHCDpurify system
- Pipettes, 1000 µL, 100 µL, 10 µL
- Real-time PCR System
- Microplate and microtube shaker

2. Methods

■ Experiment preparation

Before first use of the kit:

- Add 20 mL of Anhydrous Ethanol to Wash buffer A (NND015).
- Prepare a 70% Anhydrous Ethanol buffer with ultra-pure water in a clean tube, label as Wash buffer B.
- Store Wash buffer A&B at room temperature (RT) properly to prevent evaporation by expiration date.

Before each use of the kit:

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

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

- Place the Magnetic particles (NND030) at room temperature for 10 minutes, vortex for 5 seconds to mix thoroughly before use.

  Note: Magnetic particles can be aliquoted in advance according to the experimental requirements to avoid temperature fluctuations, which may reduce their binding capacity.

■ Samples preparation

- Sample dilution

  Test samples from the upstream process may contain high level DNA fragments that are above the upper limit of quantitation for the residual DNA assay. Please dilute the samples with ultra-pure water before DNA extraction. You may also dilute the extracted samples with Dilution buffer (NND022).

- Sample dissolution

  If the sample is a dry powder, it needs to be dissolved with the Dilution buffer or other buffer to prepare a high-concentration sample solution, then diluted with Dilution buffer before the subsequent steps.

- pH requirement

  Most samples processed downstream should maintain a neutral pH, if the pH of the sample is < 5.0 or > 9.0, 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.

- 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.

- Extraction Recovery Control (ERC)

  Extraction Recovery Control (ERC) is used to evaluate the recovery and

accuracy of DNA extraction, the performance of method validation and assay performance. The amount added to the sample is recommended to be 2 to 10 times of the amount quantified in the unspiked sample.

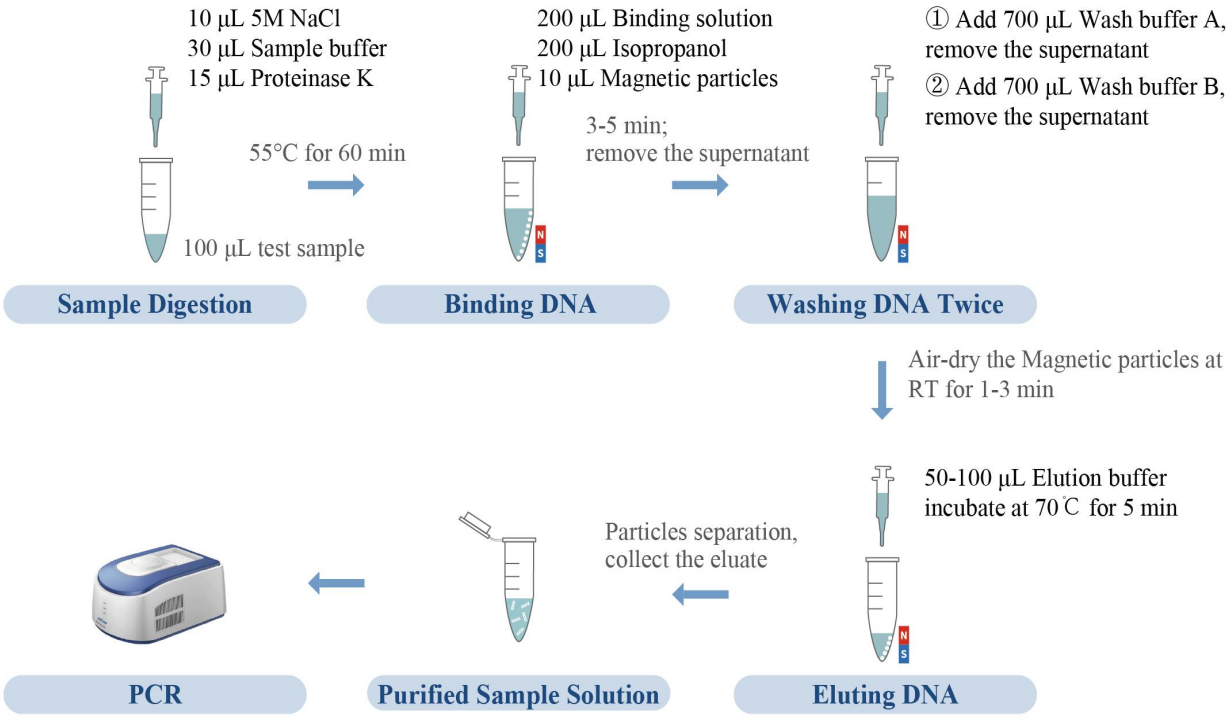
Note: For the preparation of NSC and ERC, please refer to the corresponding SHENTEK® host cell DNA qPCR detection kit user guide.

■ Sample digestion

1. Add 100 µL sample and 10 µL 5M NaCl, 30 µL Sample buffer (NND029) into a 1.5 mL microcentrifuge tube, vortex for 30 seconds to mix well, and centrifuge breifly for 5 seconds.
2. Add 15 µL Proteinase K (NND023), vortex for 10 seconds to mix well, then centrifuge breifly for 5 seconds, and incubate at 55℃ for 60 min.

■ DNA extraction

1. Manual extraction



Binding DNA

For each sample tube:

1) Place the Magnetic particles (NND030) at room temperature for 10 minutes, vortex for 5 seconds to mix thoroughly before use.
2) Remove the sample from the dry bath, and centrifuge breifly for 30 seconds, then add 200 μL of Binding solution (NND017). Vortex for 5 seconds to mix thoroughly.
3) Spin briefly for 10 seconds in a microcentrifuge, then add 200μL Isopropanol and 10 μL of Magnetic particles.

   Note: 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.
4) Vortex the tubes at medium speed for 5 minutes to bind the nucleic acids. Centrifuge for 10 seconds. Place the tubes on the magnetic stand with Magnetic particles pellet facing the magnet.
5) Wait until the solution is clear and the particles are fully separated (about 3-5 minutes). Carefully remove the supernatant without disturbing the magnetic particles.

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. Carefully remove the supernatant without disturbing the magnetic particles.
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. Carefully remove the supernatant without disturbing the magnetic particles.

3) To remove supernatant completely, spin for 10 seconds in a centrifuge 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 particles at room temperature for 1-3 minutes 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 Elution buffer (NND019), vortex for 3 seconds and incubate at 70℃ for 7 min. Vortex 2–3 times during incubation to ensure complete resuspension.

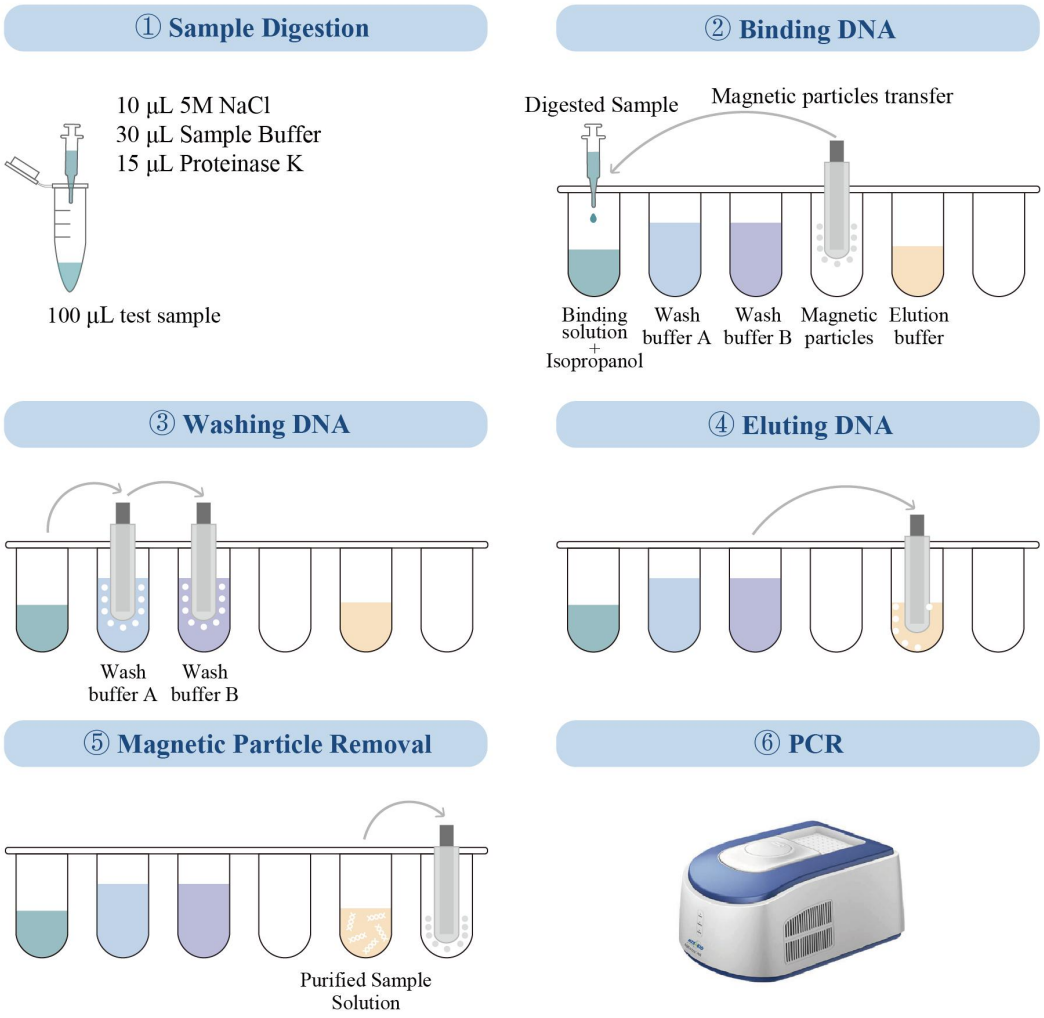
Note: Vortex the Magnetic particles and Elution buffer thoroughly. If Magnetic particles and Elution buffe stick to the tube cap or wall, briefly centrifuge again.

2) After incubation, centrifuge for 30 seconds, and then place the tubes in the magnetic stand. Wait until the particles completely separate, carefully transfer the eluate to a clean microcentrifuge tube.

3) Quickly spin the tube obtained in the previous step for 10 seconds and then place it in the magnetic stand again. Carefully transfer the eluate to a 1.5 mL microcentrifuge tube to obtain the purified sample solution.

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

2. Automated procedure with rHCDpurify system



Preparation for Extraction

During digestion, add the corresponding solution according to Table 2:

Table 2. 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: Binding solution 200 μL/well, Isopropanol 200 μL/well

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: Elution buffer 100 μL/well

Note:Add samples to column 1 or 7 after transferring all reagents.

Program setting

1) Power button on→click "login" to enter account and password→enter the main page.
2) 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.

3) Place the sample 96 deep well plate in a fixed position in the instrument and insert the plastic sleeve into the corresponding position.
4) Click "Run"→select "rHCD-06DM50" program→scan the two-dimensional code on the reagent kit→program running.
5) 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 a new 1.5 mL microcentrifuge tube.

■ Precautions

1. *It is recommended to divide the laboratory into separate areas, including the negative area (for preparation of negative control samples, PCR reagents, and negative test controls), the positive area (for sample preparation), and the amplification area, etc. Each area should be clearly marked and equipped with independent sets of equipment and supplies to prevent cross-contamination. Experimental reagents, test samples, and PCR amplification products should be stored separately and not in the same storage location. Avoid unnecessary movement within the laboratory to reduce the risk of contamination.*
2. *Before starting the experiment, ensure that the ambient temperature is not lower than 22°C.*
3. *During the experiment, select appropriately sized gloves and change them regularly. Use different lab coats, masks, hair covers, and gloves in different experimental areas to avoid cross-contamination.*
4. *Centrifuge the tubes before opening to avoid contamination of gloves or pipettes. Handle tubes with care to prevent splashing.*
5. *Dispose of used tips and liquid waste according to the regulations.*
6. *After PCR amplification, wear disposable gloves to remove the PCR tubes or plates. Check whether the caps or seals are tightly closed and whether the walls are cracked. Discard in the designated area; do not open the caps.*
7. *During the process of separating magnetic particles using the magnetic stand, rotate the tubes slowly to accelerate the aggregation of magnetic particles.*
8. *During DNA washing and eluting, ensure that no magnetic particles or liquid remain on the tube caps or walls; if so, perform a brief centrifugation.*
9. *When removing residual ethanol, avoid over-drying, which may cause incomplete dissolution during the eluting process.*
10. *Please perform DNA detection on the same day after DNA extraction to ensure accuracy.*
11. *Before starting the rHCDpurify program, check whether the 96-well deep plate and the sleeve are properly secured.*
12. *Before and after running the rHCDpurify program, UV sterilize the machine*

for at least 15 minutes and clean the inner walls with 75% ethanol wipes. The minimum interval between two extractions should be 30 minutes.

13. *Immediately after the rHCDpurify program, transfer each sample solution to a new microcentrifuge tube. Condensation on the walls of the 5th or 11th row wells will not affect DNA extraction; simply transfer the bottom eluate to a new tube and ensure that more than 40 µL of eluate is collected for the assay.*

■ 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 particles are attached too tightly to the tube walls during the eluting.	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 until the magnetic particles are suspended in the eluate.
	Low ion concentration in the sample	Adjust the ionic concentration with 5M NaCl.
	Sample pH is out of range	Adjust the pH of sample to neutral.
	Store the Magnetic particles at -20°C causes the performance drop	Store the magnetic particles at 2-8°C.

Effective date: 20 Jun. 2025

Support & Contact

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