SHENTEK

Residual Host Cell DNA Sample Preparation Kit (2G) User Guide

Version: A/0
For Research Use Only
Product No.: 1104198
Reagents for 100 Extractions

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

1. Product information

■ Product description

The SHENTEK® Residual Host Cell DNA Sample Preparation Kit (2G) can stably and efficiently extract and purify trace amounts of host cell DNA from various samples during the manufacturing process of biological products, especially for samples collected from anion exchange columns, and also for bulk, in-process samples and final products.

In addition, the kit is capable of manual sample preparation as well as automated extraction with the rHCDpurify® system, providing flexibility to meet different laboratory needs and workflows.

When combined with SHENTEK® host cell DNA qPCR detection kits, the SHENTEK® Residual Host Cell DNA Sample Preparation Kit (2G) offers a seamless, integrated workflow from sample preparation to quantitative detection. This comprehensive approach ensures accurate and consistent results throughout the entire process.

■ 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	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 × 2 bottles	
II	Magnetic particles	NND030	750 µL × 2 tubes	2-8℃
III	Proteinase K	NND023	500 µL × 4 tubes	-20℃
	Precipitation solution I	NND003	25 µL × 2 tubes	
	Precipitation solution II	NND004	500 µL × 2 tubes	

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

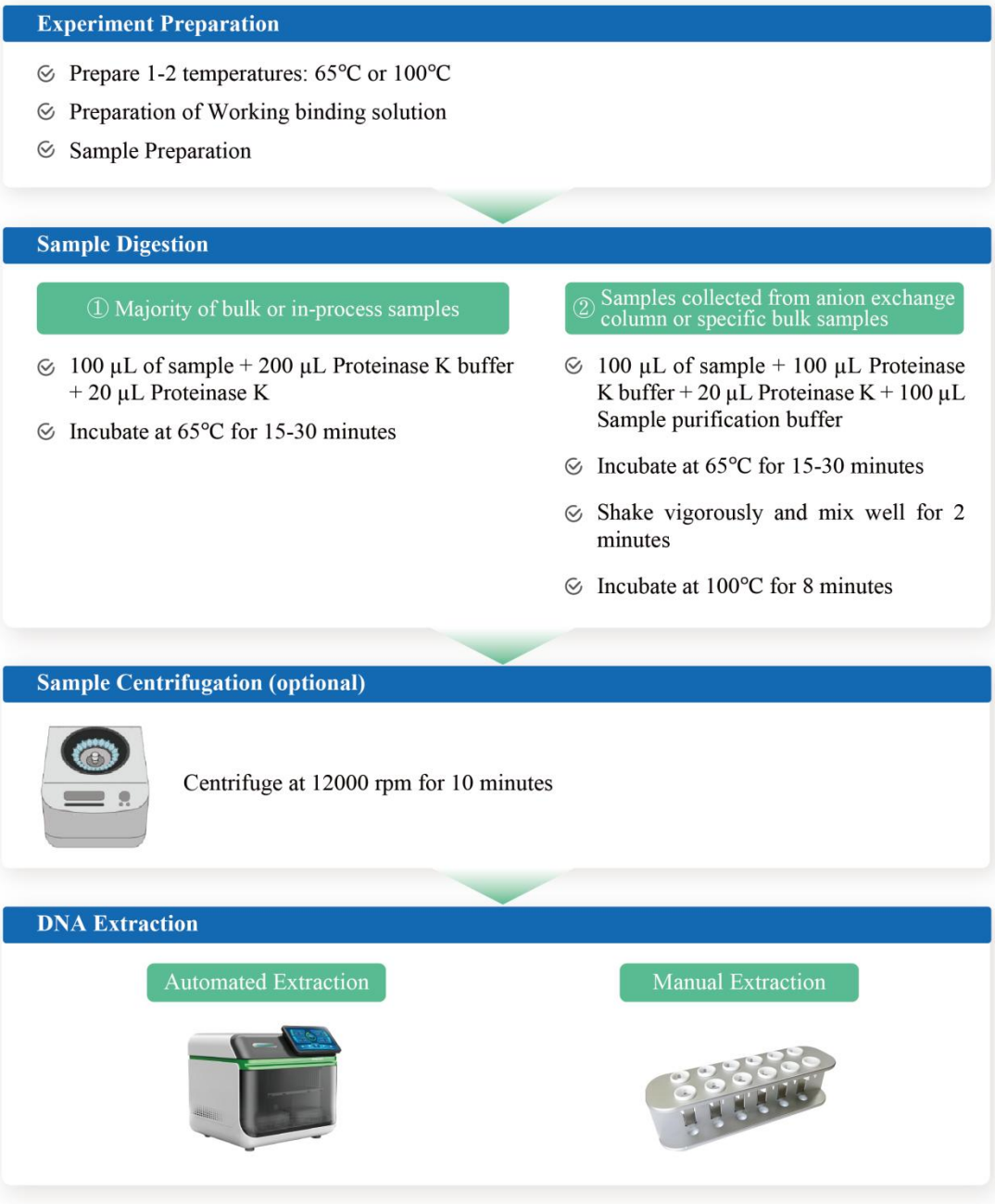
■ Required materials not included in the kit

- Anhydrous Ethanol (AR)
- 100% Isopropanol (AR)
- Low retention, sterile filter tips: 1000 µL, 100 µL, 10 µL
- Low retention, RNase/DNase-free, sterile microcentrifuge tubes, 1.5 mL
- Sample Purification Buffer (Product No.: 1104200, if necessary)

■ Related equipment

- Benchtop microcentrifuge
- Vortex mixer
- Dry bath incubator
- Magnetic stand or rHCDpurify® system
- Pipettes, 1000 µL, 100 µL, 10 µL
- Laminar flow cabinet

■ Workflow



***Note**: The above operation process is for reference only, and can be adjusted and verified according to the actual situation.*

2. Methods

■ Experiment preparation

1. Before first use of the kit:

(1) Add 40 mL of Anhydrous Ethanol to Wash buffer A (NND014).

(2) Prepare a 70% Anhydrous Ethanol buffer with ultra-pure water in a clean tube, label as Wash buffer B.

(3) Store Wash buffer A & B at room temperature (RT) properly to prevent evaporation by expiration date.

(4) Sample Purification Buffer (if necessary): Pour 1 bottle of Proteinase K Buffer (NND026) into the bottle containing the Sample Purification Reagent (NND091), tighten the cap, and mix upside down, label as Sample Purification Buffer.

Note: *The Proteinase K Buffer and the Sample Purification Reagent are the components of Sample Purification Buffer (Product No.: 1104200).*

2. Before each use of the kit:

(1) Prepare 100% Isopropanol.

(2) Set the dry bath temperatures to 65°C or 100°C.

(3) Prepare the required Working binding solution according to the following ratios:

Working binding solution for a single sample = 200 μL Binding solution (NND016) + 0.4 μL Precipitation solution I (NND003) + 10 μL Precipitation solution II (NND004).

Note: *The total volume of Working binding solution required for this experiment must be calculated and prepared according to the number of the samples. If crystallization or precipitation is found in the Binding solution before use, heat at 37 °C until it clears, then vortex to mix well.*

(4) Magnetic particles (NND030) equilibration: Remove the particles from the refrigerator at 2-8°C, fully mixed in a vortex mixer for 5-10 seconds, centrifuge briefly and leave at room temperature for at least 10 minutes to

equilibrate the particles.

3. Samples preparation:

(1) If the test sample is an upstream intermediate from the purification process of a biological product, the residual DNA content should be relatively high. To ensure the accuracy of the test results, it is necessary to keep the detection values in the linear range of the standard curve. Generally, we recommend considering diluting 100- or 1000-fold of samples with high DNA content. If the sample is diluted, use the Dilution buffer (NND021) or your selected dilution buffer as a negative control.

(2) If the sample is in dry powder form, make sure the dry powder sample can be dissolved with Dilution buffer or your selected dilution buffer before next step. Or first dissolve the dry powder sample with appropriate reagents, as a high concentration solution, and then dilute it with a dilution buffer before proceeding to the next step. We recommend diluting dry powder samples to a concentration between 10 -100 mg/mL.

(3) Sample processing: To ensure the accuracy of the results, three DNA extractions are recommended for each sample.

(4) Negative Control Sample (NCS): Each experiment requires the NCS as a blank sample prepared in the same procedure as unknown test samples, to evaluate whether there is cross contamination or environmental contamination during sample processing.

(5) Extraction Recovery Control (ERC): ERC is used to evaluate the recovery and accuracy of DNA extraction, the performance of assay validation and system condition. The concentration of ERC is recommended to be 2-10 times that of the unspiked sample.

■ Sample digestion

The following sample digestion process is for reference only, and can be adjusted and verified according to the actual situation.

1. Prepare the corresponding Proteinase K digestion solution according to the sample type.

(1) Majority of bulk or in-process samples

Proteinase K digestion solution required for a single sample = 200 μL Proteinase

K Buffer + 20 μL Proteinase K

***Note**: The actual amount of Proteinase K digestion solution can be adjusted according to the protein concentration in the test sample. The usage of Proteinase K and its buffer can be adjusted according to the following table:*

Table 2. Proteinase K Digestion Solution Required for Single Sample

Protein Concentration（mg/mL）	*pH Range*	*Proteinase K（μL）*	*Proteinase K Buffer（μL）*
0 -100	*5-9*	*10*	*100*
0-200	*＜5 or＞9*	*20*	*200*
100-200	*5-9*		

(2) Samples collected from anion exchange column or specific bulk samples

Proteinase K digestion solution required for a single sample = 100 μL Proteinase K Buffer + 20 μL Proteinase K + 100 μL Sample Purification Buffer.

Note:

① The total volume of Proteinase K digestion solution required for this experiment must be calculated and prepared according to the number of samples.

② If crystallization or precipitation is found in the Proteinase K Buffer before use, heat at 37°C until it clears, then vortex to mix well.

③ Sample Purification Buffer is a solid-liquid mixture, and needs to be mixed thoroughly before use.

2. Aliquot the Proteinase K digestion into clean 1.5 mL microcentrifuge tubes, then add 100 μL of test sample or ERC sample to the microcentrifuge tubes, vortex to mix well, and centrifuge briefly.
3. Digest the above mixture in a 65℃ dry bath for 15-30 minutes.

   ***Note:** After digestion of samples collected form anion exchange column or specific bulk samples, vortex the mixture vigorously for 2 minutes, then heat at 100℃ in a dry bath for 8 minutes.*
4. Cool down the sample to room temperature, centrifuge at 12000 rpm for 10

minutes at room temperature, and transfer all the supernatant to a new 1.5 mL microcentrifuge tube for later use.

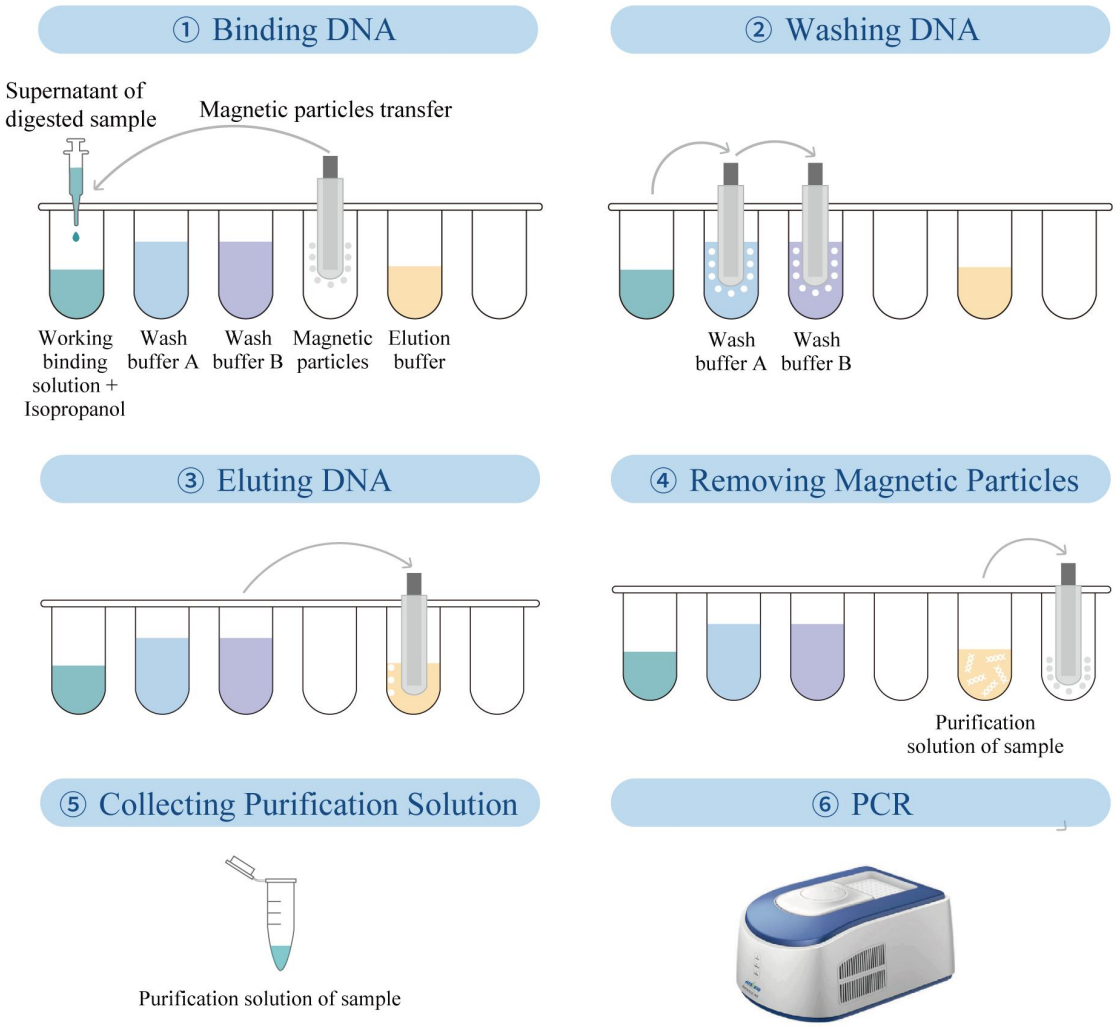
Note:

① *For in-process samples, skip the centrifugation step based on the actual situation.*

② *For samples collected from anion exchange column and bulk samples, please follow the centrifugation steps according to the description above.*

■ DNA extraction

✧ Automated extraction with rHCDpurify system



1. 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						S1-ERC					
S2						S2-ERC					
S3						S3-ERC					
S4						S4-ERC					
S5						S5-ERC					
S6						S6-ERC					
NCS											

Column 1 or 7: Working binding solution 210.4 μL/well, Isopropanol 500 μL/well and supernatant of digested sample

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 (NND018) 60 - 100 μL/well

Note:

① Magnetic particles should be mixed well by shaking or in the vortex mixer for 5 seconds before use. If there are many samples, the magnetic particles should be fully vortex-mixed during each pipetting to ensure a consistent number of magnetic particles is added each time.

② If the Proteinase K digestion solution required for single sample is prepared as 10 μL Proteinase K + 100 μL Proteinase K Buffer for sample digestion, then the volume of isopropanol added to column 1 or column 7 should be 400 μL per well.

2. 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-198” program→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.

3. Precautions

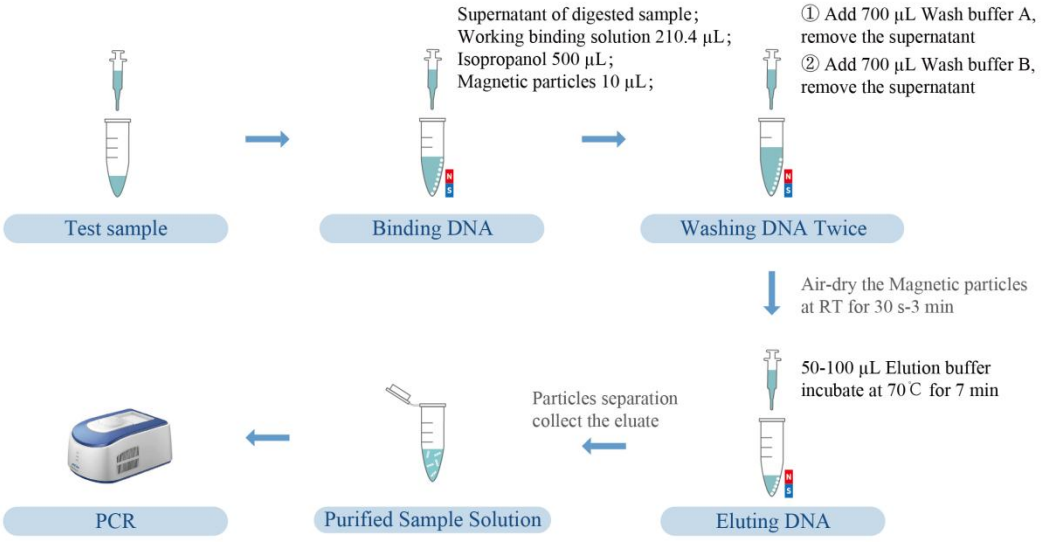
1) Before starting the rHCDpurify program, check whether the sleeves are properly secured.

2) Before and after running the rHCDpurify program, UV sterilize the machine for at least 15 minutes. The minimum interval between two extractions should be 30 minutes.

3) After the procedure is running, the purified sample is immediately transferred to a clean microcentrifuge tube. To ensure the accuracy of the test results, try to perform follow-up DNA testing on the day of sample purification.

4) Before starting the experiment, ensure that the ambient temperature is not lower than 22°C.

✧ Manual extraction



Binding DNA

For each sample tube:

1) Add 210.4 μL of Working binding solution to the supernatant of the above sample, and vortex to mix well.
2) Spin briefly for 10 seconds in the microcentrifuge, then add 500 μL of Isopropanol and 10 μL of Magnetic particles.

Note:

① Magnetic particles should be well mixed by shaking or in the vortex mixer for 5 seconds before use. If there are many samples, the magnetic particles should be fully vortex-mixed during each pipetting to ensure a consistent number of magnetic particles is added each time.

② If the Proteinase K digestion solution required for a single sample is prepared as 10 μL Proteinase K + 100 μL Proteinase K Buffer for sample digestion, then the volume of isopropanol should be 400 μL per tube.

3) Vortex the tubes 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.
4) 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:

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 on 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 on the magnetic stand. Carefully remove the supernatant without disturbing the

magnetic particles.

3) To remove supernatant completely, spin for 10 seconds in a microcentrifuge and place the tubes on the magnetic stand. Wait until the particles are completely separated, carefully remove the remaining liquid with a 10 μL pipette.

   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 – 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 of pre-warmed Elution buffer at 70℃ along the centrifuge tube wall, vortex for 5 seconds and incubate at 70℃ for 7 minutes. Vortex 2–3 times during incubation to ensure complete resuspension.

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

2) After incubation, centrifuge for 1 minute, and 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) Briefly spin the tubes obtained from the previous step for 10 seconds, and place them on the magnetic stand again. Carefully transfer the eluate to a fresh 1.5 mL microcentrifuge tube to obtain the purified sample solution.

Note: *To ensure the accuracy of the test results, try to perform follow-up DNA testing on the day of sample purification.*

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Support & Contact

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