SHENTEK

Animal-derived Biomaterial Residual DNA Sample Preparation Kit User Guide

Version: A/0
For Research Use Only
Product No.: 1104193
Reagents for 50 Extractions

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

1. Product information

■ Product description

SHENTEK® Animal-derived Biomaterial Residual DNA Sample Preparation Kit utilizes magnetic particle separation technology to extract residual DNA from a broad range of animal-derived biomaterials. This kit is suitable for the efficient and reproducible recovery of residual DNA in complex sample matrix, including but not limited to extracellular matrix, collagen and bio-absorbable membrane. The extracted DNA allows for downstream applications of residual DNA quantitation using qPCR or PicoGreen analysis.

This kit is compatible with manual sample preparation or automated extraction with rHCDpurify instrument.

■ 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	Wash buffer A	NND015	15 mL × 1 bottle	room temperature
	Binding solution	NND017	10 mL × 1 bottle	
	Elution buffer	NND020	20 mL × 1 bottle	
	1×PBS	NND042	5 mL × 1 bottle	
	DEPC-treated H_2O	NND045	5 mL × 1 bottle	
	Proteinase K Buffer	NND025	5 mL × 1 bottle	
II	Magnetic particles	NND030	750 μL × 2 tubes	2-8℃
III	Proteinase K	NND023	500 μL × 2 tubes	-20℃
	Glycogen	NND035	500 μL × 1 tube	
	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 this kit

- Anhydrous Ethanol (AR)
- 100% Isopropanol (AR)
- PCR 8-well strip tubes with caps or 96-well plates with seals
- Nonstick, Low Retention Tips: 1000 µL, 100 µL and10 µL
- Nonstick, RNase-free microcentrifuge tubes 1.5 mL

■ Related equipment

- Benchtop microcentrifuge
- Analytical balance (0.0001 g)
- Vortex mixer
- Dry bath incubator
- Magnetic stand, or rHCDpurify system
- micropipettes, 1000 µL, 100 µL and 10 µL
- Real-time PCR System

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 ddH_2O in a clean tube, label Wash Buffer B.
- Store Wash buffer A&B at RT properly to prevent evaporation before expiration date.

Before each use of the kit:

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

- Proteinase K digestion solution preparation (for one sample):

  20 μL Proteinase K + 100 μL Proteinase K Buffer + 80 μL DEPC-treated H_2O

  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 (NND025) is cloudy or contains precipitates, heat at 37°C until it clears, and mix well.

  The amount of proteinase K used can be adjusted according to the digestion efficiency.

  The effect of Proteinase K digestion will influence DNA recovery and detection.

- Binding buffer preparation (for one sample):

  200μL Binding solution + 9μL Glycogen + 0.2μL Yeast tRNA*****

  **** Do not add Yeast tRNA when using PicoGreen DNA quantitation assay.***

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

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

■ Samples 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 (1×PBS) as blank sample prepared in the same procedure as unknown test samples. The NCS will help 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.

A pilot study is recommended to confirm the amount of residual DNA in the samples, as well as the dilution factor of sampling volume.

■ Sample digestion

For each sample tube:

1. Samples need to be weighed and recorded accurately, cut into the smallest size, and place into 1.5 mL microfuge tube.
2. Add Proteinase K digestion solution, vortex to mix well and incubate at 55℃ for 60 min in dry bath incubator (Samples are completely digested without visible particles).
3. Manual extraction or machine extraction can be selected following individual procedures below.

Note: For those samples treated with cross-linking agents that are not easily digested by enzymes, please homogenize thoroughly and place into a clean 1.5 mL microfuge tubes. For wet samples, weight the same amount of test sample and record the wet weight, and calculate the moisture content according to the dry weight after it was completely dried to constant weight. Keep the moisture content for subsequent data analysis.

■ DNA extraction (Manual)

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

Binding DNA

For each sample tube:

1. Add the Binding buffer (the Binding buffer should not contain Yeast tRNA for PicoGreen DNA quantitation assay), vortex to mix well.

2. Quickly spin for 10 seconds in a microcentrifuge, then add 200 μL Isopropanol and 30 μL Magnetic particles.

Note: Before use, vortex the magnetic particles for 5 seconds until the particles are completely suspended. Magnetic particles must be resuspended before adding to each sample to ensure consistency of magnetic particles added to each sample.

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

Note: Centrifuge 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: The time for complete separation of clear solution and particles 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 (binds 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 on the magnetic stand. Discard 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. Discard 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 volume micropipette.

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

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

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

Eluting DNA

For each sample:

1. Add 100-150 μL pre-warmed (70°C) Elution buffer, vortex for 5 seconds and incubate at 70°C for 7 min. Vortex 2–3 times during incubation to ensure complete resuspension of the magnetic particles.

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

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

3. Quickly spin the tube for 10 seconds and then place it on the magnetic stand again. Carefully transfer the eluate to a fresh

nonstick 1.5 mL microfuge tube and label with the corresponding sample name.

Note: Transfer the eluate completely and avoid leaving any residuals behind. The eluent can be diluted appropriately when the concentration of the eluent exceeds that of the standard curve.

Precautions

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

2. While holding the microfuge tube in left hand, gently open the cap with your thumb without splashing.

3. Place the tubes on 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 be resuspended 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.

■ Machine extraction (rHCDpurify system)

1. Plate preparation

1. 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						PCS					

- Column 1 or 7: binding buffer 209.2 μL/well, isopropanol 200 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 30 μL/well
- Column 5 or 11: Elution buffer 100 μL/well

2. Program startup

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% alcohol cotton ball → 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 "rHCD-193" 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 microfuge tube.

3. Important points to note

- *Before starting the program, it is compulsory to add plastic sleeve.*
- *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.*
- *After the program is completed, the sample eluate needs to be transferred immediately to a clean 1.5 mL tube.*
- *Please try to conduct subsequent testing on the same day of sample DNA extraction to ensure an accuracy test results.*
- *Ensure that the ambient environment temperature not lower than 22°C during the experiment begins.*

■ 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 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, otherwise 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 resuspended 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 ntil the magnetic particles are resuspended 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.
	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 -20℃ storage of magnetic particles causes decreased performance	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 on the magnetic stand, then carefully transfer the eluate to a fresh tube.

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Support & Contact

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