ResiDNA Hunter Residual DNA Sample Preparation Kit

RD101

Instruction for Use

Version 22.1

Vazyme

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01/Product Description

ResiDNA Hunter Residual DNA Sample Preparation Kit is suitable for the pretreatment of various biological samples derived from CHO cells. It has a uniquely embedded superparamagnetic silicon-based magnetic beads with the special buffer, which can adsorb nucleic acid by hydrogen bonds and electrostatics. That could stably and efficiently extract trace amounts of host cell DNA remaining in biological samples.

02/Components

	Components	RD101-01 (100 rxns)
	Binding Buffer	40 ml
	Proteinase K Buffer	10 ml
	Wash Buffer	20 ml
	Elution Buffer	10 ml
	Magnetic Beads	6 ml
■ (blue)	Proteinase K	2 × 750 µl
■ (green)	Glycogen	2 × 500 µl
■ (red)	Yeast tRNA	50 µl

Binding Buffer: Provides the necessary environment for nucleic acid binding to magnetic beads.
Proteinase K Buffer: Provides the necessary environment for Proteinase K digestion.
Wash Buffer: Removes impurities such as salt ions.
Elution Buffer: For elution of bead-bound nucleic acids.
Magnetic Beads: For binding nucleic acids.
Proteinase K: For protein digestion in samples.
Glycogen: For stabilizing extraction efficiency.
Yeast tRNA: For reducing nucleic acid adsorption and stabilizing extraction efficiency.

03/Storage

The Proteinase K, Glycogen and Yeast tRNA should be stored at -30 ~ -15℃ and transported at ≤0℃. The other components should be stored at 15 ~ 25℃ and transported at room temperature.

04/Applications

The kit is suitable for the pretreatment of residual DNA detection in relevant biological product samples expressed by CHO cell line.

05/Self-prepared Materials

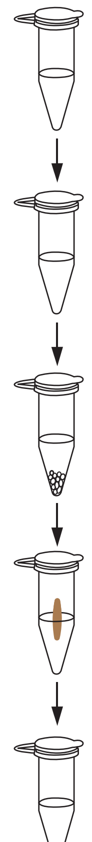
Reagents: Absolute ethanol, Isopropanol (100%), 1 M HCl, 1 M NaOH, 5 M NaCl, 1 × PBS buffer (pH 7.4).
Consumables: Low retention pipette tips, 1.5 ml Nuclease-free centrifuge tube, Magnetic Stand.
Equipment: High-speed centrifuge, Thermostat water bath, Vortexer, Microcentrifuge.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. Before the first use, please **add the specified volume of absolute ethanol** to the Wash Buffer, as described on the bottle. Store the reconstituted Wash Buffer at room temperature in the shade.
2. Vortex to mix the magnetic beads thoroughly before use.
3. Do not over-dry the magnetic beads, otherwise the nucleic acid will not be easily eluted.
4. During the operation, in order to prevent the liquid from splashing and affecting the test results, please open the tube lid gently.
5. After mixing the sample, centrifuge the tube briefly to collect the liquid to the bottom of the tube.
6. When the DNA is purified, please detect it in time. If the detection cannot be performed, the purified DNA should be stored at -85 ~ -65℃.

07/Mechanism & Workflow



Preparation:

◇Prepare Proteinase K working solution

◇Prepare Binding working solution

◇Sample preparation: refer to **08-1/Preparation**

Sample lysis:

◇ Add **80 μl Proteinase K working solution and 100 μl sample** to be detected in sequence, and incubate at 56℃ for 30 min.

Purify nucleic acid:

◇ Bind nucleic acid: Add **360 μl Binding working solution, 400 μl isopropanol, and 60 μl magnetic beads** in sequence, and vortex at room temperature for 5 min. Then centrifuge the tube at 12,500 rpm (15,000 × g) for 25 sec, place the tube in a magnetic stand and remove supernatant.

◇ Impurity removal: Add **300 μl Wash Buffer** to the tube, and centrifuge at 12,500 rpm (15,000 × g) for 25 sec, place the tube in a magnetic stand and remove supernatant. Repeat the operation once.

◇ After rinsing, incubate at room temperature for 1 - 3 min with lid open.

Elute nucleic acid:

◇ Add **50 μl Elution Buffer** to the tube and incubate at 70℃ for 7 min. Then centrifuge the tube at 12,500 rpm (15,000 × g) for 3 min, place the tube in a magnetic stand and pipette 40 μl supernatant.

Fig 1. Workflow of ResiDNA Hunter Residual DNA Sample Preparation Kit

08/Experiment Process

08-1/Preparation:

1. Prepare 56℃ and 70℃ water baths.

2. Prepare 100% isopropanol.

3. Prepare Proteinase K working solution.

Components	Volume (for single reaction)
Proteinase K	10 μl
5 M NaCl	10 μl
Proteinase K Buffer	60 μl

4. Prepare Binding working solution.

Components	Volume (for single reaction)	
Yeast tRNA	0.2 μl	■
Glycogen	9 μl	■
Binding Buffer	380 μl	

5. Sample processing:

◇ (Optional) Sample dilution: When the detected sample contains a relatively high concentration of host DNA residues, in order to ensure that the detected value of the sample is within the linear range of the standard curve, the 1 × PBS could be used to dilute the sample, and the recommended dilution ratio is 100-fold or 1,000-fold.

◇ pH adjustment: Ensure that the pH of the biological sample is neutral, it is recommended to adjust with 1 M NaOH or 1 M HCl.

◇ Negative Extraction Control (NEC): NEC should be set in parallel for each experiment (1 × PBS is recommended). The NEC is processed simultaneously with the samples to be detected to assess whether there is cross-over or environmental contamination during sample processing.

◇ Extraction Recovery Control (ERC): ERC should be set in parallel for each experiment to evaluate the reliability of the verification analysis method and system performance. It is recommended to add a DNA standard of known content in 1 × PBS. The amount of DNA standard added is set at 2 - 10 fold of sample.

08-2 Sample digestion:

1. Add 80 μl Proteinase K working solution to a 1.5 ml Nuclease-free centrifuge tube, and then add 100 μl the sample to be detected (real sample/NEC/ERC).
2. Close the cap and mix the sample with Proteinase K working solution by vortexing. Briefly centrifuge the 1.5 ml tube to collect the liquid to the bottom of the tube.
3. Incubate the mixture at 56℃ for 30 min. If the protein concentration of the sample is too high, the incubation time can be appropriately extended, but not more than 1 h.

08-3 Nucleic acid purification:

1. Take out the centrifuge tube and wait it cool to room temperature. Centrifuge the tube briefly to collect the liquid to the bottom of the tube.
2. Add 360 μl Binding working solution, 400 μl isopropanol, and 60 μl magnetic beads to the centrifuge tube in sequence. Close the cap and mix by vortexing at room temperature for 5 min.

   ▲Make sure the beads are well mixed before use.

3. Centrifuge the tube at 12,500 rpm (15,000 × g) for 25 sec at room temperature. Place the tube in a magnetic stand for 10 - 30 sec, and carefully remove the supernatant when the solution is clear.

   ▲ The suction speed should be fast first and then slow to avoid the loss of nucleic acid caused by suction of the magnetic beads, which will affect the accuracy of the results.

4. Take out the tube from the magnetic stand, add 300 μl Wash Buffer, close the lid and vortex for 10 sec. Centrifuge the tube at 12,500 rpm (15,000 × g) for 25 sec at room temperature. Place the tube in a magnetic stand for 10 - 30 sec, and carefully remove the supernatant when the solution is clear.

5. Repeat step 4.

6. Open the lid and dry for 1 - 3 min. The magnetic beads should not be over-dried. The elution efficiency will be reduced when the magnetic beads are over-drying.

7. Add 50 μl Elution Buffer along the wall of the tube, then close the lid and resuspend the magnetic beads by vortexing. Centrifuge the tube briefly to collect the liquid to the bottom of the tube, and incubate at 70℃ for 7 min.

8. Centrifuge the tube at 12,500 rpm (15,000 × g) for 3 min at room temperature. Place the tube in a magnetic stand, and carefully pipette 40 μl the product into a 1.5 ml Nuclease-free centrifuge tube. The extracted DNA can be used for subsequent detection. Please store it at -85 ~ -65℃.

9. Downstream quantitative detection can be performed with the ResiDNA Precise Quantitative CHO DNA Detection Kit (Vazyme #RD102).

08-4 Automated extraction process (automatic nucleic acid extraction instrument (Vazyme #VNP-32P))

1. Refer to 08-1/Preparation, prepare the Proteinase K working solution and the Binding working solution for the corresponding number of samples.

2. Manually dispense the specified volume of each component reagent to the corresponding position of the 96-well deep-well plate according to the table below.

96-well deep-well plate reagent dispensing scheme

Location (Column)	Components	Volume
1/7	Proteinsae K working solution	80 μl
2/8	Wash Buffer	300 μl
3/9	Wash Buffer	300 μl
4/10	—	—
5/11	—	—
6/12	Elution Buffer	70 μl

3. Add 100 μl sample to column 1/7 of the 96-deep well plate and run the RD101 pre program.

Procedure	Column	Step name	Mixing time (min)	Magnetic time (sec)	Waiting time (min)	Volume (μl)	Mixing speed	Temperature (℃)	Mixing position	Mixing range	Magnetic position	Magnetic speed
1	1	Digestion	0	0	30	180	5	56	0%	80%	0%	1

4. After the program runs, take out the 96-well deep-well plate. Add 360 μl Binding working solution, 400 μl isopropanol and 60 μl magnetic beads to column 1/7 of the 96 deep-well plate in sequence.

5. Put the 96 deep-well plate into the corresponding position of the instrument again and run the RD101 program.

RD101 program

Procedure	Column	Step name	Mixing time (min)	Magnetic time (sec)	Waiting time (min)	Volume (μl)	Mixing speed	Temperature (℃)	Mixing position	Mixing range	Magnetic position	Magnetic speed
1	1	Binding	5	180	0	1000	5	37	30%	80%	0%	5
2	2	Washing 1	0.5	60	0	300	5	-	0%	80%	0%	5
3	3	Washing 2	0.5	60	1	300	5	-	0%	80%	0%	5
4	6	Elution	5	60	0	70	8	80	0%	80%	0%	5
5	3	Discard magnetic beads	0.5	0	0	300	10	-	0%	80%	0%	1

Temperature settings: temperature control and operation synchronization, elution well preheating advance steps (1 - 3): 2

Magnetic attraction method: reciprocating magnetic attraction.

6. After the run, pipette 40 μl product from column 6/12 of the 96-well deep-well plate into a new centrifuge tube for downstream assays or freeze at -85 ~ -65℃.

09/FAQ & Troubleshooting

Observation	Possible cause	Action
Poor extraction efficiency	Sample pH is too acidic or too alkaline	Adjust sample pH to neutral with 1 M NaOH or 1 M HCl
	Too much protein in the sample	Properly extend the sample digestion time
	Magnetic beads were not mixed	Mix the beads thoroughly before use
	No absolute ethanol was added to the Elution Buffer	Before the first use, add the specified volume of absolute ethanol according to the information on the reagent bottle label
	Magnetic beads lost during binding and rinsing	Extend the magnetic suction time on the magnetic stand, and discard the liquid after the liquid is completely clarified
	Ethanol residue after washing	Aspirate residual liquid with 10 μl pipette tip
	Magnetic beads were dried for too long	The magnetic beads should not be dried for more than 3 min
	During elution, it was not ensured that the magnetic beads were all collected by brief centrifugation to the bottom of the tube	Before incubating at 70℃, ensure that the Elution Buffer and magnetic beads are at the bottom of the tube
	Residue of magnetic beads	To prolong the centrifugation time after elution, gently handle the centrifuge tube
Wealthy extraction efficiency	Cross-contamination between samples	Please open the caps from low-concentration to high-concentration samples in order, and ensure that the centrifuge tube is tightly capped after the operation

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