Instructions for Use of Host Cell Residual DNA Sample Pretreatment Kit (Magnetic Bead Method)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-CL100

Introduction

This kit is used for the pretreatment of biological product samples and accurate extraction of host cell residual DNA in various biological products. This kit is applicable to a variety of matrix buffer solutions for effective extraction and purification of trace amounts of DNA. It can be used in combination with DNA (qPCR) detection kit for biological host cells (CHO, E.coli, Vero, Human, plasmid, SV40LTA & EIA, etc.) developed by Hillgene.

Specification:

100 tests.

Main components:

Serial Number	Components	Specification	Storage conditions
1	Lysis buffer	9 mL × 1 bottle	2- 8 ℃
2	Wash buffer	30 mL × 1 bottle	
3	Eluent	12 mL × 1 bottle	
4	Magnetic beads	1 mL × 2 vials	
5	Proteinase K	1 mL × 1 vial	
6	Glycogen	800 μL × 2 vials	-20℃ or below
7	Yeast tRNA	50 μL × 1 vial	

Shelf life:

Twelve (12) months at specified storage conditions.

Equipment and reagents required for test but not provided

- Absolute ethanol (analytically pure), 1 × PBS buffer (without Mg^{2+} and Ca^{2+}, sterile filtration), isopropanol (analytically pure)
- Vortex mixer, magnetic stand, centrifuge, water bath/metal bath, pipette
- 1.5 mL sterile low-attachment centrifuge tubes, low-retention tips, disposable gloves

Test procedures

Test preparation

1. Prepare fresh 80% ethanol with absolute ethanol and sterilized ultrapure water in clean reagent bottles for each test.
2. Preparation of binding buffer for individual samples: 9 μL of glycogen + 0.2 μL of yeast tRNA (if yeast DNA is extracted, yeast tRNA will not be added to the binding buffer). Note: Single aspiration volume shall not be less than 3 μL.
3. Wash buffer preparation: Add 30 mL of absolute ethanol before using the wash buffer, mix well before use, and label properly. Cap the bottle tightly after each use to maintain the content of absolute ethanol in the bottle.

Sample Preparation

1. Sample dilution: Test samples may contain high DNA content if they are upstream intermediate samples in the purification process of biological products. To ensure the accuracy of detection and ensure that the detection value of samples is within the linear range of the standard curve, samples with high DNA content can be diluted with 1 × PB S in an appropriate ratio before sample extraction. Generally, samples with high DNA content can be diluted by 100 folds or 1000 folds.
2. Dry powder samples: In general, it is feasible to dilute the dry powder sample to a concentration at 10 mg/mL or 100 mg/mL, and then perform extraction.
3. pH requirement: Adjust the pH of the sample to neutral (pH 6.0 to 8.0) using sodium hydroxide or hydrochloric acid for extraction.
4. Parallel treatment: DNA extraction in triplicate is recommended for each sample.
5. Sample spiking: It is appropriate that the spiked DNA concentration of the test sample is 2 – 10 times the nucleic acid concentration of the sample, and it is recommended that the spiked sample volume is not more than 1/10 of the test sample.
6. Negative Control: Template-free dilution (1 × PBS) should be processed with the test sample for each test.

Operating procedures

1. Transfer 100 μL of each sample to be processed in a 1.5mL centrifuge tube for further extraction.
2. Add 25 μL of lysis buffer and 10 μL of proteinase K to each 100 μL of sample, vortex for 30 s, centrifuge briefly , and incubate at 65℃ for 15 min.

Extraction

1. After the end of incubation, centrifuge the tubes instantly, followed by the addition of 9.2 μL of binding buffer, 50 μL of lysis buffer, and 150 μL of isopropanol, and 20 μL of beads (beads should be thoroughly mixed before use to ensure that the amount of beads added each time is consistent to avoid inconsistent DNA yield), vortex for 5 min, and rapidly centrifuge for 10 s.

2. Place the centrifuge tubes on the magnetic stand and gently rotate from the left to the right for several times. After the magnetic beads are aggregated onto the wall close to the magnetic stand, fix the centrifuge tubes on the magnetic stand, remove the supernatant with a pipette, and pay attention not to touch the magnetic beads.

3. Add 500 μL of wash buffer (check that absolute ethanol has been added before use) and vortex for 30s to ensure that the beads are dispersed and there are no aggregated beads on the wall of the centrifuge tube. After rapid centrifugation for 10s, place the centrifuge tubes back on the magnetic stand and gently rotate from the left to the right for several times. After the magnetic beads are aggregated onto the wall close to the magnetic stand, allow the tubes to stand for 1 min, and carefully remove the supernatant with a pipette.

4. Add 500 μL of freshly prepared 80% ethanol and vortex for 30s to ensure that the beads are dispersed and there are no aggregated beads on the wall of the centrifuge tube. After rapid centrifugation for 10s, place the centrifuge tubes back on the magnetic stand and gently rotate from the left to the right for several times. After the magnetic beads are aggregated onto the wall close to the magnetic stand, allow the tubes to stand for 1 min, and carefully remove the supernatant with a pipette.

5. To ensure that the residual ethanol is pipetted as much as possible, the centrifuge tubes can be quickly centrifuged for 10 s and then placed on a magnetic stand to pipette the residual ethanol with a 10μL pipette.

6. Remove the tube cap and dry at room temperature for 3-5 min (the drying time may be extended or shortened depending on specific circumstances; observe visually from time to time, so as to avoid excessive drying of magnetic beads).

Note: Excessive drying of beads or presence of residual ethanol during drying will have adverse impact on the sample recovery. Drying by blast may also be selected, and it is generally recommended to dry by blast for 2 min.

7. Remove the centrifuge tubes from the magnetic stand, and add 100 μL of eluent to each tube, vortex for 1 min and incubate at 70℃ for 7 min, during which vortex every 2-3 min to mix well.

8. Following the end of incubation, centrifuge the tubes at high speed for 1 min, then allow the tube to stand on a magnetic stand and carefully transfer the supernatant to a new 1.5mL centrifuge tube using a pipette after the magnetic beads have separated.

Precautions

1. Before the test, it is recommended to wipe the surfaces of the operating table, pipettes, tip boxes, etc. with alcohol to remove the contamination.

2. It is important to change tips in a timely manner between different sample addition steps to avoid cross-contamination.

3. Equilibrate all reagents to room temperature before performing the test.

4. During bead washing or elution, it is necessary to centrifuge quickly for a short time after each shaking to ensure that no bead solution is adhered onto the centrifuge tube cap and wall.

5. To ensure the accuracy of test results, follow-up DNA testing should be conducted immediately upon the completion of sample pretreatment and purification on the same day.

6. Reagents should be stored under defined conditions and mixed use of kit reagents from different lots is not recommended.

7. The final test results are closely associated with the validity of reagents, the operation methods of operators and the

8. test environment.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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