Instructions for Use of Mycoplasma 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-CL200

Introduction

The kit is used for pre-processing of biological product samples to accurately extract residual DNAs of host cells in various biological products. The kit is applicable to various matrix buffers to effectively extract and purify trace amounts of DNAs. It can be used together with mycoplasma DNA detection kit manufactured by Hillgene.

Specification

100T.

Kit components

No.	Components	Specification	Storage conditions
1	Lysate	12 mL × 1 vial	2 – 8 ℃
2	Wash buffer	30 mL × 1 vial	
3	Eluent	12 mL × 1 vial	
4	Magnetic bead	1 mL × 2 tubes	
5	Proteinase K	1 mL × 1 tube	
6	Binding liquid	12 mL × 2 vials	
7	Glycogen	800 μL × 2 tubes	Stored at -20℃ and below
8	Yeast tRNA	50 μL × 1 tube	

Shelf life

12 months under specified storage conditions

Self-prepared instruments and reagents required for test

Absolute ethanol (AR), 1 × PBS buffer (Mg2+ and Ca2+ free, sterile filtration, pH 7.4), isopropanol (AR), 5M NaCl, 1M NaOH and 1M HCl

Vortex, magnetic separation rack, mini centrifuge, high-speed centrifuge, water bath/metal bath and pipette

1.5 mL sterile low adsorption centrifuge tubes, low adsorption tips and disposable gloves

Test procedures

- Test preparation

1. Prepare fresh 80% ethanol with absolute ethanol and sterilized ultrapure water in a clean reagent bottle for each test.
2. Preparation of working conjugate buffer for an individual sample: 200 μL of conjugate buffer + 9 μL of glycogen + 0.2 μL of yeast tRNA (if yeast DNA is to be extracted, yeast tRNA shall be not added to the conjugate buffer).

3. Preparation of wash buffer: Add 30 mL of absolute ethanol to mix well before the use of wash buffer, and make a label at the same time. Cap the bottle tightly after each use to maintain the content of absolute ethanol in the bottle.

4. Preparation of 5M NaCl: Weigh 1.4625 g of NaCl, add 5 mL of sterilized ultrapure water and shake well. It is recommended to be dispensed for storage.

5. Preparation of water bath/metal bath: 65℃ and 70℃.

- Sample preparation

1. Sample dilution: If the sample to be tested is an upstream intermediate sample in the purification process of a biological product, it may contain a high content of DNAs. To ensure the accuracy of detection and make sure that the detection value of the sample is within the linearity range of the standard curve, the sample with high DNA content can be diluted with 1× PBS buffer at an appropriate proportion before sample extraction. Generally, the sample with high DNA content can be diluted by 100 folds or 1000 folds.

2. Dry powder samples: It is generally considered to dilute the dry powder sample to 10 mg/mL or 100 mg/mL before extraction operation.

3. pH requirements: Adjust the pH of the sample to neutral (pH 6.0 ~ 8.0) using 1 M NaOH or 1 M HCl for extraction.

4. Parallel processing: three DNA extractions are recommended for each sample.

5. Sample spiking: The DNA spiking concentration of the sample to be processed shall be 2-10 folds of the nucleic acid concentration of the sample as appropriate, and it is recommended that the sample spiking volume should not exceed 1/10 of the volume of the sample to be processed.

6. Negative control: A no-template-control diluent (1× PBS buffer or DNA diluent) is required to be processed together with the sample to be tested in each test.

Operation procedures

1. Pipette 100 μL of each sample to be processed in 1.5 mL centrifuge tubes for further extraction.

2. Add 10 μL of 5 M NaCl, 100 μL of lysate, and 10 μL of proteinase K to each 100 μL sample, vortex for 30 s, instantaneously centrifuge, and incubate at 65°C for 1 h.

Note: The usage of proteinase K and lysate required for a single sample needs to be adjusted according to the sample protein concentration. See the following table for details.

Sample protein concentration	Usage of proteinase K	Usage of lysate
0-50mg/mL	10μL	100μL
50-100mg/mL	20μL	200μL

The following operation steps are divided into manual extraction and machine extraction, and the user can select the corresponding extraction method for operations as needed.

- Manual extraction

3. Instantaneously centrifuge the centrifuge tubes after incubation, add 209.2 μL of working conjugate buffer, 250 μL of isopropanol and 20 μL of beads successively (the beads need to be fully mixed before use to ensure that the volume of beads added each time is consistent to avoid inconsistent DNA yield), vortex for 10 min, and rapidly centrifuge for 10 s.

4. Place the centrifuge tubes in the magnetic separation rack and gently turn left and right until the beads gather on the walls close to the magnetic separation rack, keep the centrifuge tubes fixed in the magnetic separation rack, pipette the supernatant and pay attention not to touch the beads.

5. Add 500 μL of wash buffer (please check whether absolute ethanol has been added before use), and vortex for 30 s to ensure that the beads are dispersed and there are no beads gathering on the walls of the centrifuge tubes. Rapidly centrifuge for 10 s and place the centrifuge tubes in the magnetic separation rack again and gently turn left and right until the beads gather on the walls close to the magnetic separation rack, let stand for 1 min and then pipette the supernatant.

6. Add 500 μL of freshly-prepared 80% ethanol, and vortex for 30 s to ensure that the beads are dispersed and there are no beads gathering on the walls of the centrifuge tubes. Rapidly centrifuge for 10 s and place the centrifuge tubes in the magnetic separation rack again and gently turn left and right until the beads gather on the walls close to the magnetic separation rack, let stand for 1 min and then pipette the supernatant.

7. To ensure that residual ethanol is pipetted as much as possible, rapidly centrifuge the centrifuge tubes for 10 s and place them in the magnetic separation rack to aspirate the residual ethanol with a 10 μL pipette.

8. Open the tube caps and dry at room temperature for 3-5 min (the drying time is prolonged or shortened according to specific circumstances; observe with naked eyes at all times to avoid excessive drying of beads).

Note: Too dry beads or residual ethanol during the drying process will impact sample recovery. Blast drying may also be selected for drying, and 2-min blast drying is generally recommended.

9. Remove the centrifuge tubes from the magnetic separation rack, add 100 μL of eluent to each tube, vortex for 1 min, incubate at 70°C for 7 min, and vortex to mix well every 2-3 min during the incubation.

10. After incubation, centrifuge the centrifuge tubes at high speed for 1 min, and then let stand in the magnetic separation rack. After beads are separated, carefully pipette the supernatant to a new 1.5 mL centrifuge tube.

- Machine extraction (the following is for reference only, and it is required to combine with the specific operation process of an automatic nucleic acid extraction instrument)

3. Instantaneously centrifuge the centrifuge tubes after incubation. Add the corresponding solutions in advance according to the following deep 96-well plate arrangement, where:

✓ Column 3 or 9: 209.2 μL/well of working conjugate buffer, 250 μL/well of isopropanol, 20 μL/well of beads, and digested sample.
✓ Column 4 or 10: 500 μL/well of wash buffer.
✓ Column 5 or 11: 500 μL/well of 80% ethanol.
✓ Column 6 or 12: 100 μL/well of eluent.

Note: Column 3 or 9 can be added after all other columns have been added.

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
		Sample Isopropanol Working conjugate buffer Magnetic bead	Wash buffer	80% ethanol	Eluent			Sample Isopropanol Working conjugate buffer Beads	Wash buffer	80% ethanol	Eluent

4. Plug the instrument into the 220V AC power supply, and the instrument will show an initial interface after power-on and enter a self-test state after a few seconds. The whole power-on self-test process lasts for 1~2 min.

5. Enter different levels of the three-level management system according to the needs, click “Run” at the top of the window to enter the running interface, and click the UV lamp button for UV disinfection.

6. Open the front view door of the instrument, pull out the tray to the outermost side, place the loaded deep 96-well plate in the fixed position of the instrument, pay attention to its direction when placing the plate (position A1 is at left anterior), push the tray into the innermost side, insert the magnetic sleeves into the T-slot of the magnetic separation rack in turn, and close the front view door of the instrument. Click “Process Library” at the top of the window to select the corresponding program. Click “Edit” to modify parameters such as time, temperature, volume of solution during purification as needed.

7. After the program is confirmed, click “Run” at the top of the window to enter the running interface, click the button “Start” to start the process for operations, and the instrument will automatically process until it is completed.

Note: Check again whether the magnet sleeves are inserted into the T-slot on the magnetic separation rack before running.

8. Take out the deep 96-well plate and magnetic sleeves after the running is ended. Remove the eluent of the purified sample from the corresponding well and transfer to a 1.5 mL centrifuge tube.

Note: Centrifuge the nucleic acid purified solution at high speed for 3 min if there are residual magnetic beads in the eluent of the purified sample, let the centrifuge tube stand in the magnetic separation rack for 2 min, and carefully pipette the supernatant to a new 1.5 mL centrifuge tube after the solution is clear.

9. Click “Run” at the top of the window to enter the running interface, and click the UV lamp button for UV disinfection during which the front cover of the instrument should be closed. Close the instrument after disinfection.

Precautions

1. It is recommended to wipe surfaces of workbenches, pipettes, and tip boxes with a piece of cloth wet with alcohol to reduce contamination before the start of an experiment.
2. Tips shall be changed between different loading steps in time to avoid cross-contamination. Tips with filter elements are recommended for use.
3. Centrifuge: Select the centrifuge with quick speed-up and speed-down to prevent insufficient centrifugation from leading to experimental failure (potential contamination especially for aged centrifuges).
4. All the reagents should be equilibrated to room temperature before an experiment.
5. Before the first use, add a specified volume of absolute ethanol to wash buffer based on the label on the reagent bottle, mix well before use, and make a label at the same time. Cap the bottle tightly after each use to maintain the content of absolute ethanol in the bottle.
6. For each step operation, hold the EP tube clockwise with the left hand and gently open the tube cap with the thumb to avoid splashing the liquid.
7. The centrifuge tube can be appropriately turned when the beads are to be separated in the magnetic separation rack to make the beads be attached in a more concentrated manner.
8. During bead washing or elution, it is necessary to quickly centrifuge for a short time after each shaking and mixing to ensure that no bead solution attaches to the centrifuge tube cap and wall.
9. Subsequent DNA testing shall be performed immediately after sample pre-processing and purification as far as possible on the same day to ensure the accuracy of test results.
10. The reagents should be stored in the specified environment, and it is not recommended to mix reagents from different batches of kits during use.
11. The final test results are closely related to the efficacy of reagents, operator’s operation methods and 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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