Bacterial Genome quick

Extraction kit

货号： HDP408 100次

HDP408M 200次

The kit is made up	Save	100 次	200 次
Balance fluid	室温	10 ml	20 ml
Buffer PBS	室温	25 ml	50ml
Buffer RB	室温	25ml	50 ml
Combined liquid CB	室温	25 ml	50ml
Inhibitor removal fluid IR	室温	50 ml	100 ml
Rinse WB		25 ml	50ml
		Add the specified amount of ethanol as directed before first use	
Elution buffer EB	室温	15 ml	30 ml
Protease K powder (optional) 20mg/ml	-20℃	2×20mg	4×20mg
Adsorption column AC	室温	100 个	200 个
Collection tube (2ml)	室温	100 个	200 个

Storage matters:

1. Condensation solution CB or inhibitor removal solution IR may occur at low temperatures, which can help re-dissolve in a 37°C water bath for a few minutes, restore clarity and transparency, and cool to room temperature ready for use.

2. To avoid reducing activity and facilitating transportation, protease K is provided in the form of a lyophilized powder, which can be briefly centrifuged and dissolved with 1 ml of sterilized water, because repeated freezing may reduce enzyme activity, it is frozen immediately after dissolution according to the amount of each use (20 microliter), -20 degrees C storage.。

3. To avoid long-term exposure of reagents to the air to produce volatile, oxidation, pH changes, each solution should be used in a timely manner to close the lid.

Product introduction:

Unique binding fluid/protease K rapidly lysates cells and inactivated cell kernel enzymes, then genomic DNA selectively adsorptions to the silicon matrix membrane in the centrifugal column in a highly dissociated salt state, and then removes cell metabolites, proteins and other impurities through a series of fast rinsing-centrifugal steps, and finally the low-salt elution buffer removes pure genomic DNA from the silicon matrix membrane through a series of fast rinsing-centrifugal steps

Product features :

1. The silicon matrix film in the centrifugal adsorption column is all imported special adsorption film, the absorption difference between the column and the column is very small, and the repeatability is good.
2. There is no need to use reagents such as toxic phenol, nor does it require steps such as ethanol precipitation.

3. Fast, easy, single sample operation can generally be completed in 30 minutes.
4. Multiple column rinsing to ensure high purity, OD260/OD280 typical ratio of 1.7 ~ 1.9, length up to 30kb -50kb, can be used directly for PCR, Southern-blot and various digestion reactions.

Notes:

1. All centrifuge steps are completed at room temperature and the centrifuge speed needs to reach 13,000 rpm.
2. Preheat the water bath to 37 °C or 70 °C as needed before starting the experiment.
3. Isopropanol is required.
4. Bring your own 0.5M EDTA, Triton X-100 and Lysozyme (for Gram-positive bacteria).
5. Combined liquid CB and inhibitor removal fluid IR contains irritating compounds, the operation should wear latex gloves, avoid staining the skin, eyes and clothing. If contaminated with skin, eyes, use a lot of water or physiological saline rinse.
6. Elution EB does not contain chelating agents EDTA, does not affect downstream enzyme cutting, connection and other reactions. Water elution can also be used, but it should be ensured that the pH is greater than 7.5, and that the pH is too low to affect the elution efficiency. Wash DNA with water should be kept at -20C.DNA can be eluted with TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) if it needs to be preserved for a long time, but EDTA may affect downstream enzymatic shearing and can be diluted appropriately when used.

About the use of the balance fluid

1. Introduction: Nucleic acid adsorption silicone membrane column long-term placement process will react with the charge / dust in the air and affect its nucleic acid binding capacity. After pretreatment by the balance fluid, the silicone column can greatly reduce the water-repellent group of silicone film in the column and improve the binding ability of nucleic acids. This improves the efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution, if you accidentally touch it, please wash it with plenty of tap water. After use, close the cap to avoid contact with air. Save at room temperature. There may be precipitation generation during preservation, heat it to 37 degrees C to make the precipitation disappear completely.
2. How to use: Take a new silicone membrane adsorption column and install it in the collection tube, aspirate 100 μl of the equilibrium solution into the column. Centrifuge at 13,000 rpm for 1 min, discard the waste from the collection tube, and put the adsorption column back into the collection tube. At this point, the equilibration pretreatment column is completed.

   Follow the next steps.。

   The steps :(Please read the notes before experimenting)

   Tip: Before the first use, please add a specified amount of waterless ethanol to the rinse WB, fully mixed, add in time to check the box to mark the addition of ethanol, so as not to add more than once!

1 Take 0.5-2 ml of cultured bacteria (up to 2× 10^9 cells), 10,000 rpm, centrifuge for 30 seconds, as much as possible to absorb and clear, collect the bacteria.The initial treatment volume can be adjusted according to the bacterial density, cell species, and expected yield, and if the bacterial body exceeds the maximum adsorption capacity, the yield will be seriously reduced.

2 Add 200 μl of buffer PBS to resuspend, centrifuge at 10,000 rpm for 30 sec, discard the supernatant. Resuspend the cell oscillation or blow fully in the 180-l buffer RB.Note: For Gram-positive bacteria that are more difficult to break the wall, step 2 can be skipped and lysozyme added for wall-breaking treatment by adding 180 μl buffer (20 mM Tris, pH 8.0;2 mM Na2-EDTA; 1.2% Triton X-100; Lysozyme with a final concentration of 20 mg/ml (lysozyme must be dissolved in a buffer formulated with lysozyme dry powder, otherwise it will result in lysozyme inactivity) before use) at 37 °C for more than 30 min

3 Add a solution of 20 μl protease K (20 mg/ml), mix well, add 200 μl binding liquid CB, immediately vortex oscillate fully mixed, and leave for 10 minutes at 65 degrees C.

Optional steps: If there is more RNA residue and RNA needs to be removed, a solution of 20 μl RNase A (10 mg/ml) can be added before adding 200 μl binding fluid CB, oscillating well mixed and placed at room temperature for 5-10 minutes.

Balance fluid pretreatment adsorption column spare: pretreatment of silicone film adsorption column with balance fluid is a necessary step, see the previous "on the use of balance fluid."''

4 After cooling, add 100 μl isopropyl alcohol, immediately vortex oscillation fully mixed, at this time may appear floc precipitation. “In the above steps, it is very important to vortex or blow well-mixed sufficiently, mixing is not sufficient to seriously reduce the yield, if necessary, if the sample is not easy to mix when the viscosity is not easy to mix, can be vortex oscillating 15 seconds mixing”.

5 Add the previous mixture (including possible precipitation) to an adsorption column AC (absorption column into the collection tube), 13,000 rpm centrifugation for 30-60 seconds, and pour out the waste liquid in the collection tube.

6 Add 500 sl inhibitor to remove liquid IR, 12,000 rpm centrifugation for 30 seconds, discard liquid.

7 Add 600 sl rinse WB (check if waterless ethanol has been added first!)), 12,000 rpm centrifugation for 30 seconds, discarding liquid。

8 Add 600 sl rinsing liquid WB, 12,000 rpm centrifugation for 30 seconds, discard the waste liquid.。

9 Place the adsorption column AC back into the empty collection tube and centrifuge at 13,000 rpm for 2 min to remove the rinse solution as much as possible to avoid residual ethanol in the rinse solution inhibiting the downstream reaction.

10 Remove the adsorption column AC, place in a clean centrifuge tube, add 50-80 μl of elution buffer EB in the middle of the adsorption film (the elution buffer is better in the water bath at 65-70 degrees C in advance), leave at room temperature for 3-5 minutes, 12,000 rpm centrifugation for 1 minute.Re-add the resulting solution to the centrifugation column and leave at room temperature for 2 min and centrifuge at 12,000 rpm for 1 min.

The larger the elution volume, the higher the elution efficiency, if a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30 μl, and the volume is too small to reduce the DNA elution efficiency and reduce dna yield.。

11 DNA can be stored at 2-8 degrees C, and if stored for a long time, it can be placed at -20 degrees C.