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ME-tip500

Product Number: PLC500

Description

Plasmid purification packing is based on anion exchange column technology, utilizing gravity flow to efficiently purify plasmid DNA. The yield can reach 10mg (Giga), 2.5mg (Mega), 500μg (Maxi), or 100μg (Mini) of high copy plasmid DNA. And ensure that the purified plasmid DNA has low endotoxin levels. The entire purification process avoids toxic substances such as phenol, chloroform, ethidium bromide, and CsCl, reducing the harm to users and the environment. The plasmid DNA purified by plasmid purification packing and reagent kit is suitable for transfection, sequencing, labeling, cloning, and any other experimental operations.

Index	Parameter
Load capacity	360μg plasmid/g filler (note: loading depends on plasmid type)
Current Speed	1~2 mL/min
pH tolerance range	6~9
Form of Preservation	White solid powder
Save conditions	Sealed storage at 15~25°C for 2 years

Protocol

1. Preparation of buffer solution

1.1. Buffer P1, P2, P3, Buffer ER, balance Buffer, washing Buffer, and eluent Buffer need to be customized;

1.2. All water and Bufferare prepared using endotoxin free water, and it is recommended to filter them through a 0.22μ m membrane before use;

1.3. Consumables without heat source;

2. Filling of fillers

2.1. Add an appropriate amount of plasmid purification packing into the empty column with a lower sieve plate, compact it, and press it into the upper sieve plate;

Note: Add approximately 1.4g of powder filler to a 15mL empty column.

3. Sample preparation

3.1. Bacterial preparation

3.1.1. Take out the bacterial strain or select monoclonal bacterial spots from the -80°C refrigerator, inoculate them into a 4ml culture medium test tube, shake at 37°C for 8 hours at a speed of 300rpm, and after 8 hours, transfer 4ml of culture medium into 100ml of rich culture medium. Shake overnight at 37°C at a speed of 300rpm.

3.1.2. The bacterial solution cultured overnight was centrifuged at 4°C and 5000rpm for 15 minutes, the culture medium was discarded, and the precipitate was retained.

3.2. Bacterial lysis

3.2.1. Add 10ml of Buffer P1 to the bacterial precipitate and suspend the bacterial cells thoroughly.

3.2.2. Note: Please add RNase A to Buffer P1 before use, with a final concentration of 100μg/ml.

3.2.3. Add 10ml of Buffer P2 to the suspended bacterial solution, slowly mix evenly (without vigorous shaking), and perform lysis for no more than 5 minutes (to prevent genome contamination).

3.2.4. Add 10ml of Buffer P3 to the cracking system, mix slowly and evenly (without vigorous shaking), and a sheet-like precipitate appears. Centrifuge at 7000rpm for 10 minutes, then filter the supernatant using filter paper.

Note: If endotoxin removal is required, please add one tenth of the filtered supernatant volume of ER Buffer to the supernatant, usually 3ml, and incubate at 4°C for 30 minutes.

4. Sample purification

4.1. Place the pre installed column on a plastic rack, add 2*10ml of Balance Buffer, and wait for the liquid to flow out by gravity to complete the packing balance. Place the filler in the same buffer system as the target protein to provide

protection.

4.2. Add the prepared bacterial solution and wait for it to flow out by gravity.

4.3. Add 2*10ml washing buffer, add in batches according to the column capacity, and wait for gravity flow to complete.

4.4. Add 10ml elution buffer and collect the effluent using a 50mL centrifuge tube.

5. Isopropanol precipitation

Add 7ml of isopropanol to the centrifuge tube containing the eluent, mix it upside down, and centrifuge at 4°C and 11000RPM for 15 minutes to precipitate the plasmid. Slowly pour out the supernatant.

Note: Pre storing isopropanol at -20°C is more conducive to precipitation formation and improves recovery rate.

6. Ethanol cleaning

Add 10mL of 70% ethanol to the precipitate at 4°C and 11000RPM for 15 minutes. Slowly pour out the supernatant and let it stand at room temperature until ethanol evaporates.

7. Plasmid preservation

Dissolve the precipitate in an appropriate amount of endotoxin free water (or TE) to obtain the target plasmid solution. Using a spectrophotometer to measure plasmid concentration, the ratio of A260/A280 should be between 1.8 and 2.0. Use agarose electrophoresis to detect plasmid purity.