Plasmid Purification Beads

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1. Product Description

Plasmid Purification Beads is a new type of ion exchange column.Under certain conditions, when the plasmid DNA sample flow through the column under gravity, it is combined with the purification column.Under certain conditions, the plasmid DNA can be fully eluted, so as to realize the rapid purification of plasmid.No phenolic chloroform extraction is required.The purity of plasmid DNA was improved by three-step column (loading, washing and elution) to meet the requirements of transcell level.

Table 1. Characteristics of **Plasmid Purification Beads**

Item	Description
Ion exchange capacity	0.18-0.25mmol Cl^-/ml medium
Particle size (μm)	45-165
Flow rate	400-700cm/h
pH stability	2-12
Storage buffer	1X PBS containing 20% ethanol
Storage	4°C - 30°C

2. Purification Procedure

2.1 Buffer Preparation

If endotoxin-free plasmid is required, water and buffer should be prepared with endotoxin-free water, and consumables should be endotoxin-free.

It is recommended filtering the sample solution by passing them through a 0.22μm or 0.45 μm filter before use.

P1, P2 and P3 Buffers, Wash Buffer, ER Buffer and Elution Buffer need to be customized.

2.2 Sample Preparation

2.2.1Bacteria preparation

2.2.1.1 Remove the bacteria from the -80℃ refrigerator or pick monoclonal plaque and inoculate it in 4ml culture medium tube,incubate at 37℃ for 8 hours, and rotate at 200 rpm. After 8 hours, 4 ml of culture medium are transferred into 100 ml medium, 37 ℃ oscillation overnight (about 15 hours), and the rotation speed is 200 rpm.

2.2.1.2 Harvest cells from an appropriate volume of bacterial culture by centrifugation at 5,000rpm for 5min at 4℃,discard the culture medium and retain the precipitation.

2.2.1Bacteria Lysis

1) Add 10ml P1 buffer to the bacteria precipitation to fully suspend the cell.

Note: Please add RNase A to P1 Buffer before use, and the final concentration is 100ug/ mL.

2) Add 10ml P2 buffer to the suspended bacterial solution, mix it slowly and evenly (**no violent vibration**), and then perform the lysis. The operation time shall not exceed 5 minutes **(to prevent genome contamination**).

3) Then add 10ml P3 buffer to the cracking solution, mix it slowly and evenly (no violent vibration), and flake precipitation appears. The supernatant was centrifuged at 7000rpm for 10 minutes, and then the supernatant was filtered with filter paper.

2.3 Packing Columns

Here we describe the packing procedure of **Plasmid Purification Beads** to medium pressure chromatography columns.

a Loading of chromatography columns (using reservoir loading)

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

2) Close the column outlet leaving the net covered with packing buffer.

3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity.At first, the buffer should be allowed to flow slowly through the column, and then slowly increase to the final flow rate, so as to avoid the impact of hydraulic pressure on the column bed and the uneven formation of the column bed.If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) Maintatin packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6). With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.

7). Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8). Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

b Chromatography column testing

In order to check the quality of chromatography column, column efficiency test should be carried out to determine the theoretical plate number and peak asymmetry coefficient.

Elution Buffer： deionized water

Sample: 2% (v/v) Acetone solution or 1M NaCl

As shown in FIG. 1, the number of theoretical trays is calculated using the following formula： $N/m = 5.54 (V_R/W_h)^2 \times 1000/L$

The formula for calculating the peak asymmetry coefficient(A_S) is : $A_S = b/a$

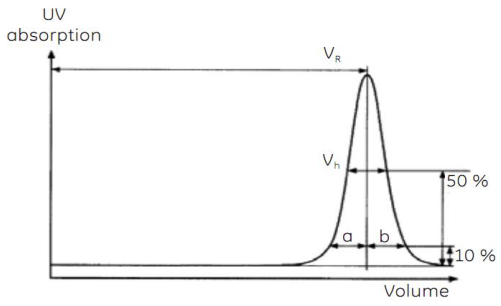

FIG.1. An example of column efficiency detection method

c Loading of a gravity column

1) Flush the bottom sieve plate and joint of the column with deionized water to ensure no bubbles on the bottom sieve plate of the column, plug the bottom outlet of the column with the lower plug, and set aside 1-2cm of deionized water at the bottom of the column.

2) The resin is suspended and the slurry is carefully poured continuously into the chromatography column.Pour slurry along the wall with a glass rod to reduce the formation of bubbles.

3) Remove the plug under the gravity column and allow the protective fluid to drain naturally.

Note: The protective fluid should not flow too dry to avoid bubbles.

2) Block the outlet of the gravity column with the lower plug.Press down the upper gasket steadily and do not exert too much force, so as not to affect the velocity of flow due to too tight packing.

Note: No bubbles should be generated during the gasket installation. In case of bubbles, the gasket should be removed, the packing should be suspended, and the gasket should be refilled.

5)Add the equilibrium liquid to the packed gravity column and begin the equilibrium.

2.4 sample purification

1) Put the medium into a suitable chromatographic column, clean the packing with 2 times of column volume of ddH_2O, balance the

packing with 2-3 times of column volume of wash buffer, and drain the wash buffer. The packing material is placed in the same buffer system as the target protein to protect the protein.

2) If endotoxin removal is required, ER Buffer should be added to the supernatant with one-tenth of the volume of the supernatant filtered in step 2.2.2, generally 3ml, and incubated at 4 ℃ for 30 minutes.

If it is not necessary to remove endotoxin,pour the supernatant filtered in step 2.2.2 into the balanced column directly to collect the outflow liquid.

3) After sample loading, wash the column with 5-10 times the volume of the column. According to the volume of the column tube, it can be added in several times to finish the wash buffer by gravity flow.

4) After washing, add elution buffer of 2-5 times the volume of column into the column tube,and use a centrifugal tube to collect the eluant.

5) Use Wash buffer (3 times column volume) and deionized water (5 times column volume) to balance the packing in turn. Finally,use 20% ethanol (5 times column volume) to balance the packing, and then store in 20% ethanol (the same volume) at 4 ℃ to prevent the packing from being contaminated by bacteria.

2.5 Isopropanol precipitation

Add 0.7 times of the volume of the eluent isopropanol into the centrifuge tube containing the eluent, mix it slowly, centrifuge to precipitate the plasmid at 11000rpm, 15 minutes, 4℃.Pour off the supernatant slowly (try not to overhang the sediment).

Note: It is recommended to add isopropyl alcohol and place it at -20℃ for 30 minutes, which is more conducive to precipitation formation and improves recovery rate.

2.6 Ethanol washing

Add 70% ethanol of one time elution liquid volume into the precipitation, centrifuge 8000rpm, 10 minutes, 4℃, slowly pour out the supernatant, place at room temperature, until the ethanol volatilized completely.

2.7 Dissolve the plasmid

Add appropriate amount of ddH_2O (or TE) to dissolve the precipitate, and then obtain the plasmid solution.The ratio of A260 / A280 should be between 1.8 and 2.0. The purity of plasmid can be detected by agarose electrophoresis.

3. Related Products

Product	Cat. No.	Size
Plasmid Purification Beads	SI014030	30 ml
	SI014100	100 ml
	SI014300	300 ml
	SI014500	500 ml
	SI01401L	1 L