DONGSHENG BIOTECH

Item No: **N1021** **10 preps**
N1022 **100 preps**
N1023 **200 preps**

Eco-Friendly plasmid miniprep kit

For research use only

Kit Content

Content	N1021	N1022	N1023
RNase A（10 mg/ml）	--	300 μl	600 μl
Solution Ⅰ	1.5 ml	15 ml	30 ml
Solution Ⅱ	5 ml	30 ml	60 ml
Solution Ⅲ	5 ml	30 ml	60 ml
Wash Buffer Q	3 ml	30 ml	30 ml×2
Wash Buffer W	3 ml×2	30 ml×2	40 ml×3
Eluent Buffer	1 ml	10 ml	20 ml
Spin Column	10	100	200

Before starting

- Add RNase A to solution I according to instructions on the label. Mix well. Mark on the label that RNase A is added. Store buffer at 4° C. Solution I of N1021 has already been premixed with RNase A, can be used directly.
- Add 1.5 volume of 96%-100% ethanol to 1 volume of Wash Buffer Q and Wash Buffer W according to instructions on each label. Mix well. Mark on the labels that ethanol is added. Store both wash buffers with ethanol at room temperature.
- If the solution II contains salt precipitates, warm the buffer in a 37° C water bath for a few minutes until precipitates dissolve. Do not shake the buffer.

Description

The Eco-Friendly system is the only method available that does not utilize Guanidine Salt, a hazardous protein denaturant, in the silica based purification system, which will ensure the safety and health of scientists and the environment.

Features

High purity: No chemicals that may cause protein degeneration; No Guanidine-HCl, CsCl, inon exchange resin, and EB; Enough for multiple applications.
High yield: 1-5ml bacterial suspension yield 10-40 μg plasmid DNA;
Time-saving: Purify your DNA in less than 30min.
Safety and environmental protection: No toxic chemicals in the solution, which is safer for people; No toxic chemicals in the waste, which is easy to dispose and good for environment.

Store

RNase A: store at -20℃. Before starting, please add RNase A to Solution I, mix well and store at 4℃.

Other reagent can be store at room temperature.

If precipitate forms in the buffers during storage, it should be redissolved by incubating the buffers at 37°C before use.

Protocol

1. Pellet 1 – 5 ml of an overnight culture (1 – 2 x 109 E. coli in LB medium). Thoroughly remove all medium from the cell pellet.
2. Completely resuspend the pellet in 100 µl Solution I with RNase A. No cell clumps should remain. Incubate the tube for 1 or 2 minutes at room temperature.
3. Add 250 µl Solution II to cells. Mix gently by inverting the capped tube 5 times. Do not vortex. Incubate the tube for 1 or 2 minutes at room temperature. Do not exceed 5 minutes.
4. Add 350 µl solution III. Mix immediately by inverting the tube until the solution is homogeneous. For large pellets shake more vigorously. White flocks will appear.
5. Centrifuge the mixture at ~12,000 rpm for 5 minutes at room temperature .
6. Load the supernatant from Step 5 into a 1.5ml tube. Add 250-300 µl ethanol (96–100%) to the tube, and mix thoroughly by inverting 3-5 times.
7. Transfer the mixture from Step 6 onto a Spin Column. Incubate for 1-2 minutes at room temperature.
8. Centrifuge at ~12,000 rpm for 1 minute. Discard the flow-through and place the column back into the Wash Tube.
9. Add 500 µl Wash Buffer Q with ethanol to the column. Incubate for 1 minute at room temperature. Centrifuge at ~12,000 rpm for 1 minute. Discard the flow-through and place column back into the Wash Tube.
10. Add 500 µl Wash Buffer W with ethanol to the column.
11. Centrifuge the column at ~12,000 rpm for 1 minute. Discard the flow-through and place the column back into the Wash Tube.
12. Centrifuge the column at ~12,000 rpm for 3 minute to remove any residual Wash Buffer W. Discard the Wash Tube with the flow-through.
13. Place the Spin Column in a clean 1.5-ml Recovery Tube. Add 75 µl of preheated Eluent Buffer to the center of the column. Incubate the column for 2 minute at room temperature. Centrifuge at ~12,000 rpm for 1 minutes. The Recovery Tube contains your purified plasmid DNA. Discard the column. Store the plasmid DNA at -20℃.

    Note: Perform all centrifugation steps at room temperature using a microcentrifuge.