Eco-Friendly Plasmid Miniprep Kit

Item N°: NB-03-0004 10 preps
NB-03-0005 100 preps
NB-03-0006 200 preps

Kit Content

Content	NB-03-0004	NB-03-0005	NB-03-0006
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.

Storage

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

Protocol

1. Pellet 1–5 ml of an overnight culture (1–2 x 10^9 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.
4. Incubate the tube for 1 or 2 minutes at room temperature. Do not exceed 5 minutes.
5. Add 250 μl solution III. Mix immediately by inverting the tube until the solution is homogeneous. For large pellets shake more vigorously. Do not vortex.
6. Centrifuge the mixture at ~12,000x g for 5 minutes at room temperature using a microcentrifuge to clarify the lysate from lysis debris.
7. Load the supernatant from Step 6 onto a Spin Column. Place the Spin Column with supernatant from Step 7 of Preparing Cell Lysate (front page) into a 2-ml Wash Tube. Incubate for 1 minute at room temperature.
8. Centrifuge at ~12,000x g 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 ~12,000x g 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 ~12,000x g for 1 minute. Discard the flow-through and place the column back into the Wash Tube.

12. Centrifuge the column at ~12,000x g 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 ~12,000x g for 1 minute. The Recovery Tube contains your purified plasmid DNA. Discard the column. Store the plasmid DNA at -20°C.

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