Plasmid Minipred Kit

Components:

Solution A	15 ml
Sloution B	15 ml
Buffer N	20 ml
Buffer W1	27 ml
Buffer W2	13 ml
Elution Buffer	5 ml
RNase A	150 μl
Spin Columns	50
Collection Tubes	50

*Absolute ethyl alcohol should be supplied by the User

Storage and Stability

The Plasmid Minipred Kit can be stored dry for up to 12 months at room temperature (15-25℃) or at 2-8℃ for storage periods longer than 12 months.

Check buffers for precipitate before use and redissolve at 37℃ if necessary. After addition of RNase A, Solution A is stable for 12 months when stored at 2-8℃. RNaseA stock solution can be stored for 12 months at room temperature.

Description

The Plasmid Minipred Kit is designed for efficient and convenient extraction of DNA. This system is based on a four-step process. A modified SDS-based DNA extraction method is applied in first step. Secondly, novel silicon matrix material is used to specific bind plasmid DNA. Thirdly, the cellular proteins, genomic DNA and RNA could be easily removed by two kinds of wash solution. Lastly, high quality plasmid DNA could be concentrated in Elution Buffer.

The Plasmid Minipred Kit could obtain 5-30 μg pure plasmids DNA from 1-4 ml culture. And the ratio of OD260/OD280 is generally between 1.8 and 2.0. Plasmid DNA prepared using the Plasmid Minipred Kit is suitable for a variety of routine applications including: PCR, enzyme digestion, sequencing, in vitro transcription, ligation and transformation.

Important notes before starting

1. Add 150 μl RNase A to Solution A before use. Mix and store at 2-8℃.
2. Add 52 ml absolute ethyl alcohol to Buffer W2 before use.
3. Check out Solution B, Buffer N and Buffer W1 before use for salt precipitation. Redissolve any precipitation by warming to 37℃. Do not shake Solution B vigorously.
4. Close the bottle containing Solution B immediately after use to avoid acidification of Solution B from CO_2 in the air.
6. Solution B, Buffer N and Buffer W1contain irritants. Wear gloves when handling these buffers.
7. All centrifugation steps are carried out at room temperature (15-25℃).

Standard DNA Purification Protocol

1. Make sure that Solution A and Buffer W2 have been prepared as described above.

2. Pellet 1-4 ml bacterial cells cultured overnight, and centrifuge at 12,000 g for 30 seconds and discard supernatant .(Remove excess media possibly.)
3. Resuspend the cell pellets in 250 μl Solution A (RNase A added). No cell clumps should be visible after resuspension of the pellet.
4. Add 250 μl Solution B and mix gently by inverting the tube 4-6 times. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 minutes.
5. Add 350 μl Buffer N and mix immediately and thoroughly by inverting tube 4-6 times. (To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N.)
6. Centrifuge for 10 minutes at 12,000 g, and keep the supernatant. Place the Spin Column into Collection Tube, and add the supernatant to Spin Column.
7. Centrifuge for 1 minute at 12,000 g and discard the flow-through.
8. Wash the Spin Column by adding 500 μl Buffer W1 and centrifuging for 1 minute at 12,000 g. Discard the flow-through.
9. Wash the Spin Column by adding 700 μl Buffer W2 and centrifuging for 1 minute at 12,000 g. Discard the flow-through.
10. Wash the Spin Column by adding additional 500 μl Buffer W2 and centrifuging for 1 minute at 12,000 g. Discard the flow-through.
11. Centrifuge at 12,000 g for additional 1 minute to remove residual wash buffer. (Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer W2 may inhibit subsequent enzymatic reactions.)
12. Place the Spin Column in a clean 1.5 ml microcentrifuge tube (not provided) and add 50-80 μl Elution Buffer (or TE PH 8.5 or water) to the center of the membrane. Incubate for 1-2 minute at room temperature and centrifuge 12,000 g for 1 minute.
13. *The volume of Elution Buffer cannot be less than 50 μl.
14. DNA recovery can be increased by using pre-warmed Elution Buffer at 60℃.

Troubleshooting guide

Low DNA yield

1. Bacterial culture is too old. Inoculate antibiotic-containing media with freshly isolated bacterial colony from an overnight plate.
2. Pelleted cells should be completely resuspended in Solution A. Do not add Sloution B until an even suspension is obtained.
3. Add elution buffer to the center of the Spin column membrane to ensure that the buffer completely covers the surface of the membrane for maximum elution efficiency.
4. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.

DNA purified does not perform well

1. RNase A is not added into Solution A or Solution A (RNase A added) is not storage at 4℃.
2. The lysate must be handled gently after addition of Solution B to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing.
3. Lysis in step 4 must not exceed 5 minutes.

4. Protein is not removed clearly. Upon addition of Buffer N in step 5, mix immediately but gently.
5. Absolute ethyl alcohol is not added into Buffer W2.
6. Eluate contains residual ethanol. Before elution , centrifuge additional 1 min at 12,000 g and incubate the spin column for 5 min at room temperature to remove ethanolic Buffer W2 completely.
7. Eluate salt concentration too high. Modify the wash step by incubating the column for 5 minutes at room temperature (15–25°C) after adding 500 µl ml of Buffer W2 and then centrifuging.