ImaSpin® Plasmid DNA Extraction Kit

For purification of plasmid DNA

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100 %)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

*Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

IP1 Buffer (Add RNase A
IP2 Buffer
IP3 Buffer
IPW1 Buffer
IW2 Buffer (Add Ethanol)
Elution Buffer
RNase A
IP Column
2 ml Collection Tubes

Storage and Stability:

This kit should be stored at room temperature but the RNase A added IP1 should be stored at 2 - 8 ˚C for up to 6 months.

Description

ImaSpin® Plasmid DNA Extraction Kit is designed by patented technology for rapid isolation of plasmid DNA from 1-5 ml of cultured bacterial cells. By using ImaSpin Buffer system, clear cell lysate with minimal genomic DNA and RNA contaminants can be obtained. In the presence of chaotropic salt, plasmid DNA in the lysate binds to the glass fiber matrix of the spin column. Contaminants are removed with a Wash Buffer (containing ethanol) and the purified plasmid DNA is eluted by a low salt Elution Buffer or TE. Typical yields

are 20-30 µg for high-copy number plasmid or 3 - 10 µg for low-copy number plasmid from 4 ml of cultured bacterial cells. Plasmid DNA can be purified in 15 minutes without DNA phenol extraction or alcohol precipitation. The purified plasmid DNA is then ready for use in restriction enzyme digestion, ligation, PCR, and sequencing reactions.

ImaSpin® Plasmid DNA Extraction Kit Test Data

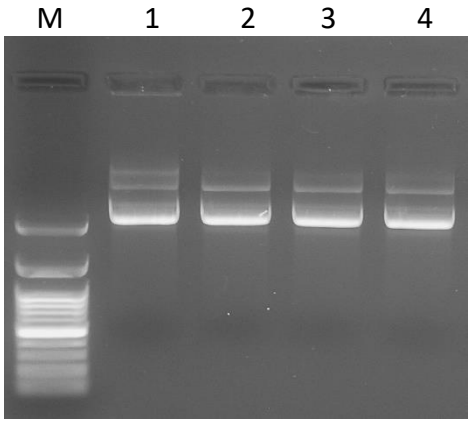

Fig 1. Plasmid DNA extraction

Plasmid DNA from E. coli (DH5α) culture, containing a plasmid pBR322 (OD600 = 3 U/ml) was extracted using the ImaSpin® Plasmid DNA Extraction Kit & Competitive brand Q. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 1 % agarose gel.
M = 1 Kb DNA Ladder
1-2 = Competitive brand Q
3-4 = ImaSpin® Plasmid DNA Extraction Kit

Preparation before using

1. Add 1 mL of IP1 Buffer to the RNase A vial and vortex for a few seconds. Transfer the solution back into the IP1 bottle and mix thoroughly. Indicate date of RNase A addition. Store IP1 Buffer containing RNase A at 2 - 8 °C. The solution will be stable at this temperature for up to 6 months.
2. If precipitates have formed in IP2 Buffer, warm the buffer in a 37 °C water bath, followed by gentle shaking to dissolve.

Protocol

1. Transfer 1.5 ml of cultured bacterial cells (3-4 x 10^9 E. coli grown in LB medium) to a 1.5 ml microcentrifuge tube.
   NOTE: Using 2 - 4 OD600 units of bacterial culture is recommended.
   And please use fresh bacterial cultures only.
2. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the harvesting step as required for samples between 1.5-5.0 ml, using the same 1.5 ml microcentrifuge tube. If the sample is more than 5 ml, please use multiple tube and extract in multiple columns.
3. Add 200 µl of IP1 Buffer (RNase A was added) and resuspend the cell pellet by vortexing or pipetting until all traces of the cell pellet have been dissolved.
4. Add 200 µl of IP2 Buffer to the resuspended sample then mix gently by inverting the tube 10 times. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.
   NOTE: Do not vortex to avoid shearing the genomic DNA.
   NOTE: After adding IP2 Buffer, any precipitates will be completely dissolved.
5. Add 300 µl of IP3 Buffer then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA.
6. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes at room temperature.
7. During centrifugation, place an IP Column in a 2 ml Collection Tube.
8. Transfer all of the supernatant to the IP Column. Use a narrow pipette tip to ensure the supernatant is completely transferred without disrupting the white precipitate.
9. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute at room temperature then discard the flow-through. Place the IP Column back in the 2 ml Collection Tube.
10. Add 600 µl of IPW1 Buffer into the IP Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds.
12. Discard the flow-through then place the IP Column back in the 2 ml Collection Tube.
13. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) into the IP Column.
14. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds at room temperature.
15. Discard the flow through then place the IP Column back in the 2 ml Collection Tube.
16. Centrifuge at 13,000 rpm (10,000 x g) rpm for 3 minutes at room temperature to dry the column matrix.
17. Transfer the dried IP Column to a new 1.5 ml microcentrifuge tube.
18. Add 50 µl of Elution Buffer or ddH2O (pH 8.0 - 8.5) directly onto the CENTER of the membrane.
    NOTE: Ensure that Elution Buffer is added into the CENTER of the IP Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60 ~ 70 °C).
19. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed.
20. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes at room temperature to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Incomplete buffer preparation.** Ensure provided RNase A was added to IP1 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. IP1 Buffer and RNase A mixture should be stored at 2 - 8 °C for up to 6 months. If precipitates have formed in IP2 Buffer, warm in a 37 °C water bath followed by gentle shaking to dissolve. Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. **Incomplete cell culture preparation.** We recommend using a single freshly isolated E. coli colony to inoculate into 1-10 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube at 37 °C with 150 - 180 rpm shaking). **Culture growth medium was not removed completely.** Following centrifugation in the harvesting step, use a narrow pipette tip to ensure the supernatant is completely removed. **Cell pellet was not resuspended completely.** Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved. **Incorrect DNA Elution step.** Ensure that Elution Buffer, TE or water is added into the center of the IP Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer, TE, or water (60 ~ 70 °C). If using water for elution, ensure the water pH is ≥ 8.0. ddH2 O should be fresh as ambient CO_2 can quickly cause acidification. **No or Low yield of plasmid DNA.** Elute twice to increase yield. Increase volume of low-copy number plasmid to 5 - 7 ml. We recommend using a single freshly isolated E. coli colony to inoculate into 1 - 10 ml of LB medium. Solid and liquid

	medium should contain antibiotics. Do not use overgrown bacterial cultures. Use fresh cultures only.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination.** Following the Wash Step, dry the IP column by incubate at 60 ˚C for 5 minutes. **Residual salt contamination.** Perform the Wash Step twice for salt sensitive downstream applications. **RNA contamination.** Ensure provided RNase A was added to IP1 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. IP1 Buffer and RNase A mixture should be stored at 2 - 8 ˚C for up to 6 months. After adding IP2 Buffer to the sample mixture, mix gently by inverting the tube 10 times then let stand at room temperature for 2-5 minutes. **Genomic DNA contamination.** Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During IP2 and IP3 Buffer addition, mix gently to prevent genomic DNA shearing.