Standard Operation Procedure

Plasmid extraction kit using spin column

Introduction:

Plasmids play a crucial role in the evolution and adaptation of bacterial cells to a changing environment, since they carry genes that have beneficial traits. In molecular biology, plasmid DNA plays a vital role in routine applications such as PCR, sequencing, cloning, transfection and manipulation of genes. Cambrian plasmid extraction and purification kit is designed for extracting and purifying plasmid DNA from recombinant gram-negative bacteria (*E. coli*).

Storage condition:

Plasmid extraction kits can be kept at room temperature. If RNAse A is being mixed with the resuspension buffer then the reconstituted resuspension buffer should be stored at 4°C up to six months.

Kit contents:

Contents	Quantity	Storage
Resuspension buffer	12.5 ml	Room temperature
Lysis buffer	12.5 ml	Room temperature
Neutralisation buffer	17.5 ml	Room temperature
Wash buffer concentrate	10 ml	Room temperature
Elution Buffer	2.5 ml	Room temperature
Cambrian Spin columns	50 no.s	Room temperature
Reconstitution buffer	0.5 ml	Room temperature
RNAse A	2 mg	Store at 4°C until reconstituted with resuspension buffer

How to start:

Items required by the user but not provided in the kit:

Equipment:

1. Table-top micro-centrifuge (with rotor for 1.5 mL and 2 mL tubes).
2. Vortex mixer.
3. Sterile Pipettes and pipette tips.

Reagents to be supplied by the user:

1. 96%- 100% Ethanol

Recommended Sample volume for starting:

1. Pellet 1-5 ml recombinant bacteria harbouring the plasmid of interest grown overnight culture by centrifuge at 13,000 rpm for 3 minutes at room temperature (15 -25°C).

Reconstitution of RNAse A with resuspension buffer

1. Add 200 ul of Reconstitution buffer to RNase A bottle and mix it thoroughly.
2. From reconstituted RNase A solution add 130 ul to the resuspension buffer and store the resuspension buffer at 4°C.

Preparation of wash buffer solution

1. Wash buffer: The wash buffer is provided as a buffer concentrate. **Add 40 ml of 96-100% ethanol to the wash buffer concentrate before use**.

*Do not use denatured alcohol which contains other undesirable substances like methyl-ethyl ketone or methanol.

Protocol

1. Resuspend the pelleted bacterial cells in **250** µl of resuspension buffer (containing RNAse A) and transfer to a nuclease free microcentrifuge tube.
2. Add **250** µl lysis buffer and mix thoroughly by inverting the tube 4-6 times. Vortex lightly till the cell pellet fully dissolves. **Do not let the lysis step continue more than 5 minutes.**
3. Add **350** µl of neutralization buffer and mix immediately by inverting the tube 4-6 times.
4. Centrifuge the tubes for 10 minutes at 13,000 rpm in a tabletop microcentrifuge.
5. Apply **750** µl of supernatant from step 4 to the Cambrian spin column provided with the kit. Centrifuge for 1 minute at 13,000 rpm. Discard the flow through
6. Add **750** µl of wash buffer to the spin column and centrifuge at 13,000 rpm for 1 min. ***Give an additional spin of 30 seconds to discard any residual wash buffer.**
7. Place the spin column in a sterile nuclease free 1.5 ml microcentrifuge tube and add 50 ul of elution buffer at the centre of the Cambrian spin column, let it stand for 2 minutes and centrifuge for 1 minute at 13,000 rpm.
8. Keep the eluted plasmid at -20 °C for long term storage.

Additional information and tips

1. Warm the elution buffer for 2 minutes at 60° C and use the elution buffer for plasmid extraction.

2. If working with a low copy plasmid (e.g. pBR322/pSC101 backbone etc.) use 5- 10 ml of culture volume for increased yield.

3. For increased yield one can use an elution volume of 70-100 ul. Please note that this will increase the yield but not the concentration.