One-step Yeast Plasmid Rapid Preparation Kit

Cat#: DP5001 50 preps

I. Kit Content Storage and Stability

Content	Storage	DP5001
Buffer YE	RT	5 ml
Buffer EB	RT	5 ml

All reagents, if stored properly, are stable for 12 months.

Reminder:

1. Buffer YE may precipitate under low temperature. Incubate in 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.
2. Keep all of the reagents lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

II. Principle

This Kit provides a simple and shortcut method for yeast plasmid extraction. Lyticase and vitreous beads are not used to ensure plasmid integrity. Utilize a series of elution-centrifugation steps will remove cellular metabolites and proteins. Plasmid DNA is eluted in a low salt and high pH buffer.

III. Features

1. lyticase and vitreous beads are not used.
2. Simple and fast. Extraction can be finished in 30 min.

IV.Notes

1. All centrifugation steps can be performed at room temperature.
2. Agarose gel electrophoresis or UV－spectrometer can be used for detecting the concentration and purity of the plasmid. The supercoiled plasmid conformations may display multiple bands at different sizes on agarose gel. These multiple bands are influenced by culture time and extracting methods.
3. Set a water bath at 60-70℃ before starting.
4. Use fresh samples to achieve high yields.
5. 95% ethanol, phenol, and chloroform are not included in the kit.

V. Procedure

1 · Harvest 1.5 ml culture by centrifugation at 14,000 rpm for 20 seconds. Discard the supernatant.

2 · Add 100µl Buffer YE to resuspend the pellet. Then add equal volume (100 µl) phenol/chloroform (1:1) mixture and vertex aggressively for 10min.

3 · Incubate at 65 ℃ for 5 minutes.

4 · Centrifuge at 14,000rpm for 5 minutes.

5 · Transfer the supernatant from step 4 to a new microcentrifuge tube and add two volumes 95% ethanol. Let it sit at room temperature for 5 minutes. Then centrifuge at 12,000rpm for 5 minutes and discard the supernatant.

6 · Wash the pellet with 70% ethanol twice. Dry 5 minutes in vacuum concentrator or air dry at room temperature. Resuspend the pellet with 100µl Buffer EB.

7 · Store plasmid DNA at -20℃ or apply to down-stream reactions.