TIANprep Yeast Plasmid DNA Kit

For fast and convenient purification of plasmid DNA from yeast

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TIANprep Yeast Plasmid DNA Kit

(Spin Column)

Cat. no. 4992436

Kit Contents

Contents	4992436 50 preps
RNase A (10 mg/ml)	150 µl
Buffer BL	30 ml
Buffer YP1	15 ml
Buffer YP2	15 ml
Buffer YP3	20 ml
Buffer PD	30 ml
Buffer PW	15 ml
Buffer EB	15 ml
Spin Columns CP2	50
Collection Tubes 2 ml	50
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Storage

TIANprep Yeast Plasmid DNA Kit can be stored dry at room temperature (15-25°C) for up to 12months without showing any reduction in performance and quality. For longer storage, these kits can be stocked at 2-8°C. If any precipitate forms in the buffers after storage at 2-8°C, it should be dissolved by warming the buffers at 37°C for 10 min. RNase A (10 mg/ml) can be stored for 12 months at room temperature (15-25°C). After addition of RNase A, Buffer YP1 is stable for 6 months at 2-8°C.

Introduction

TIANprep Yeast Plasmid DNA Kit is designed for quick plasmid DNA purification from yeast cells. It is based on alkaline lysis technology followed by adsorption of DNA onto silica membrane in the presence of high salt. Plasmid DNA purified with TIANprep Yeast Plasmid DNA Kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required.

Plasmid DNA prepared by TIANprep Yeast Plasmid DNA Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and trans-formation, *in vitro* translation, and transfection of robust cells.

Important Notes Before starting

1. Add the provided RNase A solution to Buffer YP1 **(use 1 vial RNase A per bottle Buffer YP1)**, mix, and store at 2-8°C.
2. Check Buffer BL, YP2 and YP3 before use for salt precipitation. Redissolve any precipitate by warming at 37°C for several minutes.
3. Close the bottle containing Buffer YP2 and YP3 immediately after use.
4. All centrifugation steps are carried out at 12,000 rpm (~13,400×g) in table-top microcentrifuge at room temperature (15-25°C).
5. The amount of extracted plasmid is related to cells concentration and plasmid copy.
6. Using Buffer BL to treat spin columns could activate silica membrane at maximum degree and higher yield.
7. After treated with Buffer BL, use the Spin Column soon, otherwise long-term placement may affect the purifying effect.

   Buffer not provided in kit:

   1) Lyticase (TIANGEN)

   2) Sorbitol Buffer: prepare 1.2 M Sorbitol with 0.1 M Phosphate Sodium Buffer (pH 7.4).

   e.g.: 77.4 ml 0.1 mol/L Na_2HPO_4+22.6 ml 0.1 mol/L NaH_2PO_4

Protocol

Add ethanol (96-100%) to Buffer PW before use (check bottle label for volume).

1. Column equilibration: Place a Spin Column CP2 in a clean collection tube, and add 500 µl Buffer BL to CP2. Centrifuge for 1 min at 12,000 rpm (~13,400×g) in a table-top microcentrifuge. Discard the flow-through, and set the Spin Column CP2 back into the collection tube. **(Please use freshly treated spin column).**
2. Harvest 1-5 ml yeast cells (<5×10^7) to a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400×g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15-25°C), aspirate the supernatant as much as possible (For large volume of bacterial cells, please harvest to one tube by several centrifugation steps).
3. Disruption of yeast cell wall:

   a. Enzyme digestion: Add 300 µl Sorbitol Buffer and 50 U Lyticase to the yeast cells. Mix completely. Shake at 220 rpm/min, 30°C for 1 hour. Centrifuge for 10 min at 4,000 rpm (~1500×g). Discard the flow-through. Resuspend pelleted bacterial cells in 250 µl Buffer YP1 **(Ensure that RNase A has been added)**.

   Note: The concentration and digestion time of Lyticase should be adjusted according to yeast strain type and cell number.

   b. Beads treatment: Resuspend pelleted bacterial cells completely in 250 µl Buffer YP1 (Ensure that RNase A has been added). Then add 0.1 g acid-washing beads with 0.45-0.55 mm diameter to the solution. Vortex for 10 min.
4. Add 250 µl Buffer YP2 and mix thoroughly by gently inverting the tube 6-8 times. Let it stand for 5-10 min at normal temperature.

   Note: Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. The solution should become viscous and slightly clear at this step .
5. Add 350 µl Buffer YP3 and mix immediately and thoroughly by inverting the tube 6-8 times. The solution should become cloudy at this time. Centrifuge for 20 min at 12,000 rpm (~13,400×g) in a table-top microcentrifuge.

   Note: To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer YP3. If there is still small white precipitate in the supernatant, please centrifuge again.

6. Apply the supernatant from step 5 to the Spin Column CP2 **(put the CP2 in a collection tube)** by pipetting. Centrifuge for 1 min at 12,000 rpm (~13,400×g). Discard the flow-through, and put Spin Column CP2 back to the collection tube.
7. Wash the Spin Column CP2 by adding 500 µl Buffer PD and centrifuging for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through.
8. Wash the Spin Column CP2 by adding 600 µl Buffer PW **(Ensure that ethanol (96-100%) has been added)** and centrifuging for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through, and put Spin Column CP2 back to the collection tube.
9. Repeat step 8.
10. Centrifuge for an additional 2 min at 12,000 rpm (~13,400 × g) to remove residual wash buffer PW.

    Note: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. And then, it's suggested to opening CP2 lid and putting it at room temperature for a while. Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.
11. Place the Spin Column CP2 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl Buffer EB to the center of Spin Column CP2, let stand for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g).

    Note: If the volume of eluted buffer is less than 50 µl, it may affect recovery efficiency. The pH value of eluted buffer will have a great effect on eluting, we suggest using buffer EB or distilled water (pH 7.0-8.5) to elute DNA. For long-term storage of DNA, eluting in Buffer EB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Supplement:

1. The copy number of yeast plasmid is usually very low, it's difficult to detect through electrophoresis or spectrophotometer detection. If the purified plasmid is used for following experiment, we suggest to use:

   1-5 µl plasmid as PCR template.

   5-10 µl plasmid for transformation of *E.coli*.
2. Commercialized high-efficient competent cells should be used for transforming *E.coli*.