Yeast Plasmid Miniprep Kit

(High Purity, Silica - membrane Spin Column)

Catalog No.: D1081 (50 preps) D1082 (100 preps) D1083 (200 preps)

Kit Contents

Component	D1081	D1082	D1083
	50 preps	100 preps	200 preps
RNase A (10 mg/ml)	150 µl	300 µl	600 µl
Solution YI	15 ml	30 ml	60 ml
Solution YII	15 ml	30 ml	60 ml
Solution YIII	20 ml	40 ml	80 ml
Wash Buffer PB	18 ml	36 ml	72 ml
Wash Buffer W	30 ml	30 ml × 2	40 ml × 3
Elution Buffer (10 mM Tris-HCl, pH 8.5)	5 ml	10 ml	20 ml
Spin Columns	50 each	100 each	200 each

Description

High Purity Plasmid Miniprep Kit is designed for rapid and cost-effective small-scale extraction of high quality plasmid DNA from yeast (*Saccharomyces cerevisiae*) cultures. The kit uses lyticase for highly efficient enzymatic disruption of yeast cell walls, and utilizes silica-based membrane technology in the form of a convenient spin column.

The purification theory: silica particles bound DNA selectively at high salt concentration and low pH, while protein and other impurities are removed. The pure DNA is eluted with low salt buffer or water. This method requires few manipulations, and is both faster and easier to perform than other organic-based extraction methods.

Downstream Applications

Purified DNA is free of impurities and enzyme inhibitors, and have an $A_{260/280}$=1.7-1.9, is suitable for applications such as:

PCR/qPCR/Restriction enzyme digestion/Sequencing/In vitro transcription

Features

- **Fast** - procedure takes only 30 min (not contain cell wall disruption)
- **High purity** - purified DNA without enzyme inhibitors, RNA or proteins, ready for downstream applications

Storage

Store RNase A at -20°C, other reagents at room temperature for up to 1 year.
The Solution YI with RNase A is stable for 6 months at 4°C. Any precipitate in the Solution YII can be dissolved by incubating at 37°C before use.

Important Notes

• Add all provided RNase A solution to the Solution YI and mix well.

• Prior to the initial use of the kit, dilute the Wash Buffer PB, Wash Buffer W with isopropyl alcohol or ethanol (96-100%):

Solution	D1081 (50 preps)	D1082 (100 preps)	D1083 (200 preps)
Wash Buffer PB	18 ml	36 ml	72 ml
Isopropyl Alcohol	12 ml	24 ml	48 ml
Total Volume	30 ml	60 ml	120 ml
Solution	**D1081 (50 preps)**	**D1082 (100 preps)**	**D1083 (200 preps)**
Wash Buffer W	30 ml	30 ml × 2	40 ml × 3
Ethanol	45 ml	45 ml × 2	60 ml × 3
Total Volume	75 ml	75 ml × 2	100 ml × 3

Mix well, mark the labels on the bottle that isopropyl alcohol or ethanol is added.

• Ensure that no DNases are introduced into the sterile solutions of the kit.
• Make sure there is no precipitates in Solution YII. If any precipitate is visible, warming the solution at 37°C for 3-5 min to dissolve the precipitate, and cooling to 25°C before use.
• Wear disposable gloves when handling Solution YII, Solution YIII and Wash Buffer PB as they contain guanidine hydrochloride or other denaturing agent.

• All purification steps should be carried out at room temperature.
• All centrifugations should be carried out by a table-top microcentrifuge at>1,2000 g (10,000-14,000 rpm, depending on the rotor type).

Preparation Work - Growth of Yeast Cultures

Pick well-isolated yeast colonies growing on the selective medium appropriate for the strain and plasmid (e.g., SD/-Leu if using a Matchmaker™ pGADT7 vector). Patch/spread each colony on a fresh plate of selective agar medium to create a 1 cm x 1 cm square. Incubate the plate at 30°C for 3 days to grow the patches.

Protocol

1. Harvest 1-5 ml overnight culture (≦5 x 10^7 yeast cells). Centrifuge at 12,000 rpm for 1 min. Remove the medium thoroughly.

2. Resuspend the cell pellet in 300 µl lyticase buffer and 50 U lyticase by pipetting up and down gently several times or by brief vortexing until the mixture is homogeneous. Shaking at 30°C for 1 hr in a shaking incubator gently.

Note: *Lyticase need to be supplied by the user or purchased from Bio Knowledge Lab, S.L.. The incubating time may vary for different species.*

3. Centrifuge at room temperature for 10 minutes at ~5,000 rpm. Discard the supernatant.

4. Resuspend the cells in 250 µl **Solution YI**. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
   Note *• Ensure RNase A has been added to the Solution YI.*

5. Add 250 µl **Solution YII** and mix gently by inverting the tube 4-6 times until the lysate mixture is thoroughly homogenous. Do not vortex.
   Note *• Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoil plasmid DNA.*

6. Add 350 µl **Solution YIII** and mix immediately and thoroughly by inverting the tube 4-6 times. Do not vortex.Centrifuge at 12,000 rpm for 10 min.
   Note *• The neutralized bacterial lysate is cloudy and viscous.*

7. Transfer the supernatant to the supplied spin column.
   Note *• Do not over 700 µl one time. Avoid transferring the white precipitate.*

8. Centrifuge at 12,000 rpm for 1 min. Discard the flow-through.
   Note *• Do not add bleach to the flow-through.*

9. Add 500 µl **Wash Buffer PB** to the spin column. Centrifuge at 12,000 rpm for 1 min and discard the flow-through.
   Note *• Wash Buffer PB must be diluted with isopropyl alcohol previously.*

10. Add 500 µl **Wash Buffer W** to the spin column. Centrifuge at 12,000 rpm for 1 min and discard the flow-through.
    Note *• Wash Buffer W must be diluted with ethanol (96-100%) previously.*

11. Repeat step 10 again.

12. Centrifuge at 12,000 rpm for 3 min to remove residual wash solution. Discard the collection tube with flow-through.

13. Place the spin column into a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl **Elution Buffer** to the center of the column without touching the membrane. Incubate at room temperature for 2 min and centrifuge at 12,000 rpm for 2 min.
    Note *• Prewarm Elution Buffer to 65℃ will increase the yield of DNA, especially the plasmids or cosmids > 20 kb.*
    Note *• A second elution step will recover residual DNA from the membrane and increase the overall yield by 10-20%.*

14. Discard the column. **Store the purified plasmid DNA at -20℃**.