Yeast Plasmid Extraction Kit

Cat: D1160
Size: 50T/ 100T
Storage: Store at room temperature, re-test period is 12 months. **(Note: RNase A and Yeast Lysing Enzymes will be shipped as an accessory, please store at -20℃)**
Kit Contents:

Kit Components	D1160-50T	D1160-100T	Storage
RNase A	300μL	300μL×2	-20℃
Yeast Lysing Enzymes	1.25mL	1.25mL×2	-20℃
Sorbitol Buffer	25mL	25mL×2	RT
β-Mercaptoethanol	300μL	600μL	RT
Solution YP1	15mL	30mL	RT
Solution YP2	15mL	30mL	RT
Solution YP3	20mL	40mL	RT
Washing Buffer I	24ml	48ml	RT
Washing Buffer II	15mL	15mL×2	RT
Elution Buffer	15mL	30mL	RT
Adsorption Column	50 个	100 个	RT
Collection Tube	50 个	100 个	RT

Note：Please add absolute ethanol to the washing buffer before use. Please refer to the label on the bottle for the added volume. (16mL/32mL of absolute ethanol should be added to each bottle of 50T/100T washing buffer I, and 45mL of absolute ethanol should be added to each bottle of washing buffer II). Add RNaseA to YP1 before use (Add all the RNase A provided in the kit), mix well, and store at 2-8℃. Unless specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

Introduction:

Yeast Plasmid Extraction Kit is designed to lysis the cell by alkali pyrolysis and Enzyme digestion to obtain yeast plasmid. Lysing Enzymes can destroy the cell wall efficiently, increase the yield of yeast plasmid. The silicon matrix material used in the centrifugal adsorption column can adsorb DNA efficiently and specifically, and can remove impurity proteins and other organic compounds in cells to the greatest extent. The yeast plasmid DNA extracted by this kit can be applied to a variety of conventional molecular biology experiments, including enzyme digestion, PCR, sequencing, linking and transformation tests. This kit is safe to operate without the use of toxic reagents such as phenol and chloroform.

Protocols (*only for reference*):

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1. Take 1-5mL yeast cultures (no more than 5 × 10^7 cells) in a microcentrifuge tube, centrifuge for 1min at 12,000rpm, remove the supernatant as much as possible (if there are too much yeast liquid, yeast can be collected into a centrifuge tube by multiple centrifugation).

2. Disruption of yeast cell wall:

A: Enzyme digestion: Completely resuspend the yeast precipitate in 470μL Sorbitol Buffer and mix throughly. Add 25μL Lysing Enzymes and 5μL β-Mercaptoethanol, mix throughly. Incubate at 30℃ for 1-2h. Invert the tube several times during incubating. Centrifuge for 1min at 12,000 rpm, discard supernatant. Add 250μL solution YP1 **(Ensure that RNase A has been added)**, Completely resuspend the precipitate. Note: If the mass is not thoroughly mixed, it will affect the cleavage and lead to low plasmid extraction and purity.

B: Beads treatment: Completely resuspend the yeast precipitate in 250 μL solution YP1 **(Ensure that RNase A has been added)**. Then add 150-200μL acid-washing bead (Not provided) to the solution. Vortex for 10 min. Centrifuge for short, carefully aspirate and transfer the cleared supernatant to a clean centrifuge tube. Please supplement with YP 1 to 250 μL if the supernatant have any loss.

3. Add 250μL YP2 to the centrifuge tube and gently turn it up and down 6-8 times to fully crack the bacteria. Note: Mixing must be gentle, so as not to contaminate the yeast genomic DNA, at this time the bacterial solution will become clear and sticky, and the action time should not exceed 5 minutes, so as not to damage the plasmid.

4. Add 350μL YP3 to the centrifugation tube, immediately and gently turn up and down 6-8 times, thoroughly mix, at this time will appear white flocculent precipitate. Centrifuge at 12000rpm for 20min. Use a pipette to carefully transfer the supernatant to another clean centrifuge tube, trying not to suck out the precipitation. Note: YP3 should be mixed immediately after addition to avoid local precipitation. If there is still a small white precipitate in the supernatant, the supernatant can be taken after centrifugation again.

5. Take the supernatant of the previous step, add 0.4 times the volume of absolute ethanol, and mix well (it can be can be divided twice if there are too much solution).

6. Carefully transfer the cleared supernatant to a adsorption column. Put at room temperature for 2min and centrifuge for 1min at 12000rpm, discard the waste liquid, and put the adsorption column back into the collection tube.

7. Add 600μL washing buffer I **(please check whether absolute ethanol has been added before use)** into the adsorption column, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

8. Add 600μL washing buffer II **(please check whether absolute ethanol has been added before use)** into the adsorption column, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

9. Repeat step 8.

10. Centrifuge the column at 12000rpm for 2min. Open its cap, and put at room temperature or 50℃ for several minutes to remove the rest of washing buffer in adsorption column. Otherwise, the ethanol in the washing buffer will affect subsequent experiments, such as enzyme digestion, PCR, etc.

11. Put adsorption column into a new clean centrifuge tube. Add 50-200uL Elution buffer which is

after 65℃ water bath to the center of the adsorption film, put at room temperature for 2min. centrifuge at 12000rpm for 1 min. V02

12. (Optional) In order to increase the recovery efficiency of plasmids, the eluent obtained can be re-added into the adsorption column, place at room temperature for 2min, and centrifuge at 12000rpm for 1 min.

Notes:

1. Check YP2, YP3 before use. If the buffer appears turbid, dissolve it by warming at 37℃ for several minutes, they can be used until become clear.
2. If the volume of elution buffer is less than 50uL, it may affect recovery efficiency. The pH value of elution buffer has influence in eluting too. If using water as elution buffer, pH should be controlled at 8.0 (adjusted by NaOH), pH below 7.0 will affect elution efficiency. The extracted DNA should be at -20℃ to prevent degradation.
3. The amount of extracted plasmid is related to cells concentration and plasmid copy number and culture condition. Generally, yeast plasmid copy number is very low, which is difficult to detect by electrophoresis or spectrophotometer, and can be detected by PCR or transformed E. coli.
4. DNA concentration and purity detection: the size of the extracted plasmid DNA fragments is related to the storage time of the sample, the shear force during the operation and other factors, The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak in OD260, OD260=1 is equal to 50ug/mL double-stranded DNA, 40ug/mL single-stranded DNA. OD260/OD280 should be 1.7-1.9, the value will be lower if using distilled water in eluting, because the pH and ions will affect the light absorption value, but do not show the purity is low.

Related Products:

R1020	*Yeast Lysing Enzymes*
D1900	*Yeast Genomic DNA Extraction Kit*
T1060	*TAE Buffer, 50×*
G8142	*GoldView II Nuclear Staining Dyes (5000×)*

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