TIANGEN®

EndoFree Mini Plasmid Kit

This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

EndoFree Mini Plasmid Kit

(Spin Column)

Cat. no. GDP123

Kit Contents

Contents	GDP123-02 (50 preps)	GDP123-03 (200 preps)
Buffer P1	15 ml	60 ml
Buffer P2	15 ml	60 ml
Buffer P4	15 ml	60 ml
Endotoxin Removal Buffer ER	5 ml	20 ml
Buffer ED	35 ml	140 ml
Buffer PW	15 ml	2 × 30 ml
Buffer TB	15 ml	30 ml
RNase A (10 mg/ml)	150 µl	600 µl
Spin Columns CP4	50	200
Collection Tubes 2 ml	50	200
RNase-Free Centrifuge Tubes (1.5 ml)	50	200

Storage

The kit can be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C before use. RNase A (10 mg/ml) can be stored for 15 months at room temperature (15-30°C). After addition of RNase A, Buffer P1 is stable for 6 months at 2-8°C.

Introduction

This kit uses unique silica membrane adsorption technology to efficiently and precisely bind plasmid DNA. Meanwhile, the unique solution ER can effectively remove endotoxin; the extraction process only takes 1 hour, which is convenient and fast. The following procedure is suitable for the extraction of 1-5 ml of overnight cultured E. coli. The ratio and quality of plasmid extraction are related to the host bacteria species and culture conditions, cell lysis, plasmid copy number, plasmid stability, antibiotics, and other factors.

The plasmid extracted by this kit can be used to transfect cultured cell lines and for routine operations, including restriction enzyme digestion, PCR, sequencing, ligation, and other experiments.

Yield For Reference

Plasmid type	Bacterial fluid volume	Yield	Plasmid
Low copy	1-5 ml	3-12 μg	pBR322, pACYC and its derivative vector pSC101 and its derivative vector, SuperCos, pWE15
High copy	1-5 ml	6-30 μg	pTZ, pUC, pBS, pGM-T

Notes <u>Please be sure to read this precaution before using the kit.</u>

1. Add the provided RNase A solution to Buffer P1 before use (use 1 vial RNase A per bottle Buffer P1), mix, and store at 2-8°C.
2. 100% ethanol should be added to the buffer PW before the first use according to the instructions on the label of the reagent bottle.
3. Check whether the buffer P2 and P4 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C for several minutes.
4. Be careful not to mix buffer P2 and P4 directly, and tighten the lid immediately after use.
5. All centrifugation steps are carried out at 12,000 rpm (~13,400×g) in a bench-top microcentrifuge at room temperature (15-30°C).
6. The amount of plasmid extracted is related to the concentration of bacterial culture, plasmid copy number and other factors. If the proposed plasmid is a low-copy plasmid or a large plasmid larger than 10 kb, the amount of bacteria used should be increased, and the amount of

P1, P2 and P4 should be increased proportionally, and the elution buffer should be preheated at 65-70°C. The adsorption and elution times can be extended appropriately to increase the extraction efficiency.

Reagents need to be prepared by Customer

96-100% ethanol

Protocol

Add 100% ethanol to buffer PW before use, and refer to the label on the bottle for the volume to be added.

1. Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400×g) in a bench-top microcentrifuge for 1 min at room temperature (15-30°C), remove all the supernatant.

   Note: 1.For large volume of bacterial cells, please harvest to one tube by several centrifugation step. 2.The efficiency of plasmid extraction will be lowered if too much bacteria are not lysed sufficiently.

2. Add 150 µl buffer P1 to the centrifuge tube with the bacterial pellet (check that RNase A has been added first), use a pipette or vortex to thoroughly suspend the bacterial cell pellet.

   Note: Please make sure to suspend the bacterial pellet thoroughly, if there are any pieces of bacteria that are not thoroughly mixed, it will affect the lysis and lead to low yield and purity.

3. Add 150 µl buffer P2 to the centrifuge tube and gently turn the tube up and down 6-8 times to fully lyse the organisms.

   Note: Mix gently and do not shake vigorously to avoid contamination of genomic DNA. At this moment, the bacterial solution should become clear and viscous, and the time should not exceed 5 min in order to avoid damage to the plasmid. If it does not become clear, the lysis may be incomplete due to too many organisms and the amount of organisms should be reduced.

4. Add 150 µl buffer P4 to the centrifuge tube and immediately mix well by gently turning the tube up and down 6-8 times, a white precipitate will appear, centrifuge at 12,000 rpm (~13,400×g) for 7 min, at this point a precipitate will form at the bottom of the centrifuge tube.

   Note: P4 should be mixed immediately after addition to avoid localized precipitation. If there is still a tiny white precipitate in the supernatant, the supernatant can be taken after centrifugation again.

5. Transform the supernatant to a new EP tube (provided by customer). To the supernatant, 60 µl Endotoxin Removal Buffer ER is added, and the solution took on a homogeneous, clear yellow color after mixing upside down.
6. Add 0.3 times the volume of isopropanol to the mixture (adding too much isopropanol may lead to RNA contamination), mix upside down and transfer to Spin Columns CP4 (spin columns are placed in collection tubes).

   Note: The filtrate will be lost after filtration, please add different volumes of isopropanol depending on the loss. The maximum volume of the Spin Columns CP4 is 700 µl, so it is necessary to pass the columns in batches.
7. Centrifuge at 12,000 rpm (~13,400×g) for 1 min at room temperature, pour out the waste liquid in the collection tube, and put the spin columns back into the collection tube.

   Note: The solution obtained in step 7 is passed through the column in several passes, each time following the above conditions.
8. Add 600 µl Buffer ED to Spin Columns CP4, centrifuge at 12,000 rpm (~13,400×g) for 1 min, and pour off the waste liquid in the collection tube.
9. Add 700 µl buffer PW to Spin Columns CP4 (**please check that 100% ethanol has been added first**), centrifuge at 12,000 rpm (~13,400×g) for 1 min, pour off the waste solution in the collection tube, and place Spin Columns CP4 into the collection tube.

   Note: After adding the buffer PW, it helps to better remove impurities if left at room temperature for 2-5 min.
10. Add 700 µl buffer PW to Spin Columns CP4, centrifuge at 12,000 rpm (~13,400×g) for 1 min, and pour off the waste solution in the collection tube.
11. Place Spin Columns CP4 back into the collection tube and centrifuge at 12,000 rpm (~13,400×g) for 2 min, with the aim of removing residual solution from the spin columns.

    Note: Due to the ethanol residue in the solution will affect subsequent enzyme reactions (enzyme cutting, PCR, etc.), it is recommended that the Spin Columns CP4 be uncapped and left at room temperature for a few minutes to thoroughly dry the solution remaining in the adsorbent membrane.

12. Place Spin Columns CP4 in a clean centrifuge tube, add 50-100 µl elution buffer TB dropwise to the middle of the adsorbent membrane, leave at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400×g) for 1 min to collect the plasmid in the centrifuge tube.

    Note: To increase the recovery efficiency of the plasmid, the resulting solution can be reintroduced into the centrifugation spin columns and repeat step 12. The pH of the eluent has a strong influence on the elution efficiency. If water is used as the eluent, pH should be in the range of 7.0-8.5. pH lower than 7.0 will reduce the elution efficiency. The volume of elution buffer should not be less than 50 µl, and too small a volume affects the recovery efficiency. And the DNA product should be stored at -20°C to prevent DNA degradation.

Plasmid DNA concentration and purity test

The plasmid DNA obtained can be tested for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. The electrophoresis may be a single band or 2 to 3 DNA bands, which is mainly related to the length of the extract incubation time and the degree of vigorous operation during extraction. An OD_{260} value of 1 corresponds to approximately 50 µg/ml double-stranded DNA.

The OD_{260}/OD_{280} ratio should be 1.7-1.9. If deionized water is used in the elution instead of elution buffer, the ratio will be low, but it does not indicate a low purity because the pH and the presence of ions will affect the light absorption value.