Endotoxin-Free Ultrapure Plasmid DNA Maxiprep (Catalog: TBS6037)

Catalog	Kit Size
TBS6037-10	10 Prep
TBS6037-25	25 Prep

Endotoxin-Free Utrapure Plasmid Extraction Maxiprep is composed of macroporous silica–based ion-exchange resin with a high density of diethylaminoethyl (DEAE) group, purification is based on the interaction between negatively charged phosphates of nucleic acids and positively charged DEAE groups on the surface of the resin, which could efficiently exclude all impurity during the binding-washing-elution process and yield ultrapure plasmid DNA.

Our powerful endotoxin-removal reagent EF Reagent can effectively remove lipopolysaccharides from bacterial cell lysates by phase partition without chilling step. Simply add the EF reagent into the clear cell lysate, and the lipopolysaccharide contaminants can be removed with the flow-through. The Kit System consistently produces endotoxin level less than 0.1 EU/μg DNA of endotoxin free plasmid DNA product.

Parameter	Value
DNA Fragment length	1.5-kbp ~ 150 kbp
Maximal Capacity	>500μg
Maximal Recovery	99%
Minimal Elution Volume	10 mL
Regular Sample Volume	100 mL
Endotoxin Level	Less Than 0.1EU/ μg

Kit Components

Kit components	TBS6037-10	TBS6037-25
No. of preparations	10	25
PB1	120 mL	265 mL
RNase A Solution (20mg/mL)	0.6mL	1.3mL
PB2	120 mL	265 mL
PB3	120 mL	265 mL
PNB4	225 mL x 2	265 mL x 4
PEB5	120 mL	265 mL
EF Reagent	8 mL	20 mL
Maxi Column	10 Pieces	25 Pieces
Mini Columns	10	25

Feature

- Ion-exchange based columns for rapid and simple extraction.
- Reproducible yield of up to 500 μg of endotoxin free plasmid DNA.
- Effective endotoxin removal reagent.
- Endotoxin level less than 0.1 EU/μg DNA in the final DNA product.

Application

- Transfection of sensitive cells (mammalian primary/suspension cells).
- Gene silencing study.
- Microinjection.
- NGS.
- Gene editing.

Important Notices

1. The volume of PB1-3 Buffer used in the protocol is developed for 100ml sample culture. If the starting sample culture is larger than 100ml, please increase the volume of PB1-3 buffer proportionally.
2. Use endotoxin-free equipment, plasticware and glassware for all steps to prevent endotoxin contamination. The use of a laminar flow hood is strongly recommended.
3. All reagents & solutions not included in the kit (e.g. isopropanol, 70% ethanol, and TE buffer) should be endotoxin-free grade and freshly prepared with endotoxin-free water.

Preparation

1. Spin **RNase A Solution** tube before use. Apply all **RNase A Solution** to **PB1 Buffer** before use, mix well and store at 2-8℃.
2. If precipitates form in **PB2**, redissolve at 37℃ for 10 minutes. Do not shake PB2 Buffer since SDS in the buffer may lead to serious foaming.
3. Sit PB3 Buffer on ice before use.
4. Add 4 part of isopropanol into 1 part of EF Reagent bottle and mix completely by inverting several times, when first open.
5. Maxi Column Preparation:

5.1 Equilibrate Maxi Columns by applying 5 ml of 98% ~ 100% ethanol. Allow the column to empty by gravity flow and discard the filtrate.

5.2 Apply 10 ml of PNB4 Buffer to the Maxi Column and allow it to flow through by gravity flow and discard the filtrate.

Protocols

1. Culture plasmid-containing bacterial cell in 100-250 ml (high copy number plasmids) or 350-500 ml (low copy number plasmids) of LB medium (TBS8056). Grow 12-16 hours with vigorous shaking at 37℃.
2. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.
3. Resuspend the cell pellet in 10 ml of PB1 Buffer. The bacterial cells should be completely resuspended before adding PB2 Buffer.
4. Add 10 ml of PB2 Buffer, mix gently by rotating the lysate and stand for 5 minutes. Do not vortex, vortexing will shear genomic DNA. The lysate should be clear and viscous.
5. Add 10 ml of ice-cold PB3 Buffer, mix gently by rotating. After adding PB3 Buffer, white precipitate should be formed.
6. Centrifuge at 20,000 x g for 15 minutes at 4℃ (20,000 x g corresponds to 12,000 and 13,000 rpm in Beckman JA-17 and Sorvall SS-34 rotors, respectively)
7. Transfer the supernatant into a 50 ml conical tube and add 4 ml of EF Reagent (Isopropanol added). Invert the tube 8~12 times and stand for 2 minutes (The volume of added EF Reagent should be more than 1/10 volume of the supernatant from Step. 6.)

8. Apply the mixture to the Maxi Column and allow it to flow through by gravity flow and discard the filtrate.
9. Wash the column twice with 30 ml of PNB4 Buffer by gravity flow and discard the filtrate.
10. Apply 10 ml of PEB5 Buffer to elute DNA by gravity flow. (Using an endotoxin-free tube to elute DNA can prevent endotoxin contamination in subsequent steps.).
11. Precipitate DNA by adding 7.5 ml (0.75 volumes) of room temperature isopropanol to the elute. Mix and centrifuge at 15,000 x g for 30 minutes at 4℃. Carefully remove the supernatant, do not disturb the DNA pellet.
12. Wash the DNA pellet twice with 5 ml of endotoxin-free, room temperature 70 % ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.
13. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 250 µl or a suitable volume of endotoxin-free TE or ddH2O.
14. (Optional) To eliminate the insoluble material, load the dissolved DNA sample into a Mini Column (sitting in a 1.5 ml tube) and spin at full speed in a microcentrifuge for 30 seconds, collect the eluted DNA sample in the 1.5 ml tube.
15. 17. Store DNA at -20℃ for further use.