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BioVision
BioVision Incorporated

ToxOut™ Endofree Plasmid Maxi Kit

(Catalog # K1330-2, -10, -25; Store at multiple temperatures)

I. Introduction:

BioVision's ToxOut™ Endofree Plasmid Maxi Kit is designed for fast and efficient purification of plasmid DNA from 150-200 mL of *E. coli* culture. The maxi column has a DNA binding capacity of 1200 µg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection. Two endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA and Protocol B removes endotoxin after the purification of plasmid DNA.

Unlike other kits in the markets, our patented plasmid purification kit has no chaotropic salts in the buffer. The purified DNA is guanidine/anion exchange resin residues free. The endofree system uses a specially formulated buffer that extracts the endotoxin from the bacterial lysate. The endotoxin level is less than 0.1 EU (Endotoxin) per µg of plasmid DNA. The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 2 times.

II. Sample Type:

For fast and efficient purification of endotoxin free plasmid DNA from 150-200 mL of *E. coli* culture.

III. Kit Contents:

Components	K1330-2 2 preparations	K1330-10 10 preparations	K1330-25 25 preparations	Part Number
ezBind Columns	2	10	25	K1330-XX-1
Buffer A1	22 mL	110 mL	270 mL	K1330-XX-2
Buffer B1	22 mL	110 mL	270 mL	K1330-XX-3
Buffer N3	8 mL	40 mL	85 mL	K1330-XX-4
Buffer RET	22 mL	110 mL	270 mL	K1330-XX-5
RNase A	110 µL	400 µL	1.35 mL	K1330-XX-6
Endofree Elution Buffer	5 mL	25 mL	60 mL	K1330-XX-7
DNA Wash Buffer	25 mL	50 mL	100 mL	K1330-XX-8
Syringe Filters	2	10	25	K1330-XX-9

IV. User Supplied Reagents and Equipment:

- High speed microcentrifuge
- 70% ethanol and 100% ethanol
- 30 mL high speed centrifuge tubes

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Except the Buffer A1 (once RNase A is added), which is stored at 4°C, all other components are stored at RT. The guaranteed shelf life is 12 months from the date of purchase. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

- RNase A: It is stable for half a year under RT. Spin down RNase A vial briefly. **Add the RNase A solution to buffer A1** and mix well before use.
- Buffer B1 precipitates below RT. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation.
- In case the collection tube doesn't fit in high-speed centrifuge rotor, use benchtop centrifuge and spin at 2,500 g with double centrifugation time. For example, centrifuge at 2,500 g for 10 min instead of 5,000 g for 5 min.
- Carry out all centrifugations at RT.

VII. Endofree Maxiprep Spin Purification Protocol:

The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 2 times.

1. Inoculate 150-200 mL LB containing appropriate antibiotic with 100 µL fresh starter culture. Grow at 37°C for 14-16 hr with vigorous shaking. *Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 hr with vigorous shaking (~250 rpm). Note: Do not use a starter culture that has been stored at 4°C. Note: Do not grow starter culture directly from glycerol stock. Note: Do not use more than 200 mL culture or cell mass greater than 550. The buffer volumes need to be scaled up if processing over 200 mL of culture.*
2. Harvest the bacteria by centrifugation at 5,000 g for 10 min at RT. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium completely.
3. Add **10 mL Buffer A1** (Add RNase A to Buffer A1 before use) and completely resuspend bacterial pellet by vortexing or pipetting (complete resuspension is critical for optimal yields).

4. Add **10 mL Buffer B1**, mix gently but thoroughly by inverting 5-10 times. If necessary, continue inverting the tube until the solution becomes slightly clear. Incubate at RT for 5 min to obtain a slightly clear lysate. *Note: Do not incubate longer than 5 min. Over-incubation can cause genomic DNA contamination and plasmid damage.*
5. Add **3 mL Buffer N3**, mix immediately by sharp hand shaking for 5-10 times. Transfer the sample to a high speed centrifuge tube and centrifuge at 12,000g for 10 min at RT. *Note: It is critical to mix the solution well. If the mixture still appears conglobated, brownish or viscous, more mix is required to completely neutralize the solution. Note: Syringe filter could be used to filtrate the lysate if high-speed centrifuge is not available.*
6. Transfer 20 mL clear lysate to a clean 50 mL conical tube and add **0.5 volume of Buffer RET** (For example, **10 mL of Buffer RET** to 20 mL of clear lysate), and **10 mL 100% ethanol** mix well by sharp hand shaking for 2 times.
7. Immediately apply 20 mL of the solution into the ezbind DNA column with the collection tube. Centrifuge at >2,500 g for 2 min at RT. Remove the column from the tube and discard the flow-through liquid. Process the remaining solution, discard the flow-through liquid and put the column back to the collection tube. *Note: If the 50 mL collection tube doesn't match the rotor (for example, the lid of rotor cannot close), the column with the collection can be centrifuged at a benchtop centrifuge at >2,500 g for 5 min.*
8. Add **10 mL DNA Wash Buffer** to the column, and allow the liquid pass through the column. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step 8.
9. Centrifuge the column at 5,000 g, with the lid open for 10-20 min to remove the ethanol residues. *Note: Ethanol residues in the column will affect the elution of DNA. If the speed is less than 5,000g, centrifuge the column for 20 min and dry in the air to completely remove the ethanol.*
10. Transfer the column to a clean 50 mL tube and add **1-1.5 mL Endofree Elution Buffer** to the center of the column and incubate for 1 min at RT. Elute the DNA by centrifugation at >2,500 g for 5 min.
11. Reload the elute into the center of the column and incubate for 1 min. Elute the DNA by centrifugation at >2,500 g for 5 min. *Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, in vitro translation, sequencing, transfection, and microinjection. Note: Two elution give rise to maximum DNA yield.*
DNA concentration (µg/mL) = OD $_{260\ nm}$ X 50 X dilution factor

VIII. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low Yield	• Poor Cell lysis. • Bacterial culture. Overgrown or not fresh. • Low copy number plasmid.	• Resuspend pellet thoroughly by votexing and pipetting prior to adding Buffer B1. • Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2M NaOH and 1%SDS). • Grow bacterial 12-16 hr. Spin down cultures and store the pellet at -20°C if the culture is not purified the same day. Do not store culture at 4°C overnight. • Increase the culture volume.
No DNA	• Plasmid lost in Host *E. coli.*	• Prepare fresh culture.
Genomic DNA contamination	• Over-time incubation after adding buffer B1.	• Do not vortex or mix aggressively after adding Buffer B1. Do not incubate more than 5 min after adding Buffer B1.
RNA contamination	• RNase A not added to Buffer A1.	• Add RNase A to Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	• Ethanol traces were not completely removed from the column.	• Make sure that no ethanol residue remains in the silicon membrane before elute the plasmid DNA. Re-centrifuge again if necessary.

FOR RESEARCH USE ONLY! Not to be used on humans.