ToxOut™ Endofree ezFilter Plasmid Maxi Kit

(Cat# K1331-2, -10, -25; Store at RT)

I. Introduction:

BioVision's ToxOut™ Endofree ezFilter Plasmid Maxi Kit is designed for fast and efficient purification of plasmid DNA from 150-400 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 150-400 mL of *E. coli* culture.

III. Kit Contents:.

	K1331-2	K1331-10	K1331-25	
Components	**2 Preparations**	**10 Preparations**	**25 Preparations**	**Part Number**
ezBind Columns	2	10	25	K1331-XX-1
Filter Syringe (60 mL)	2	10	25	K1331-XX-2
Buffer A1	22 mL	120 mL	260 mL	K1331-XX-3
Buffer B1	22 mL	120 mL	260 mL	K1331-XX-4
Buffer N3	8 mL	100 mL	130 mL	K1331-XX-5
Buffer RET	40 mL	120 mL	260 mL	K1331-XX-6
DNA Wash Buffer	50 mL	50 mL	2 x 50 mL	K1331-XX-7
Endofree Elution Buffer	12 mL	25 mL	60 mL	K1331-XX-8
RNase A (20 mg/mL)	110 µL	400 µL	900 µL	K1331-XX-9

IV. User Supplied Reagents and Equipment:

- Centrifuge with swing bucket rotor and centrifuge tubes
- 100% ethanol
- 50 mL conical tubes
- Vacuum manifold

V. Shipment and Storage:

All the reagents are shipped at room temperature. Except the Buffer A1 (once RNase A is added), which is stored at 4°C, all other components are stored at room temperature (22-25°C). The guaranteed shelf life is 12 months from the date of purchase. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

- RNase A: Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use. Store at 4°C.
- Buffer B1 precipitates below room temperature. 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.
- Ensure the availability of centrifuge capable of 10,000 rpm if perform centrifugation protocol.
- Ensure the availability of centrifuge capable of 5,000g if perform vacuum manifold protocol.
- Carry out all centrifugations at room temperature.

VII. <u>Vacuum Manifold Protocol:</u>

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 2-3 times. *Note: The Maxi column has a plasmid DNA capacity of more than 1.0 mg. To yield more plasmid DNA, please use more bacterial culture. The following information is suitable for high copy plasmid and the OD_{600} of culture is between 2.0-3.0. If the OD_{600} is lower than 2.0, that will reduce the yield of the plasmid DNA. Please use more culture accordingly.*

1. Inoculate **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 5,000g for 10 min at room temperature. 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). Transfer the lysate to a 50 mL conical tube.
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 room temperature for 5 minutes to obtain a slightly clear lysate. Complete lysis is critical for DNA yield.
5. Add **3 mL Buffer N3**, mix immediately by sharp hand shaking for 5-10 times. *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.*

6. Spin the sample at **5000g for 2 min** at room temperature. Tape the barrier of a syringe to a 50 mL tube (not supplied) set in a rack. Using a serological pipet to transfer most of the lysate, avoid only a small portion of the major precipitates, to the barrier of the syringe. Let it sit for 5 minutes. Gently insert the plunger to the barrier to expel the clear lysate into the tube. *Note: The cleared lysate should be clear and free of cloudy precipitates. It it looks turbid or cloudy, add 2 ml of Buffer N3, mix well and filter through another syringe filter. Alternatively, the sample can be centrifuged at 5,000 rpm for 5 min, transfer the clear supernatant to a clean 50 mL conical tube.*
7. Add **10 mL Buffer RET** and **10 mL 100% ethanol**. Mix well by vortexing for 2 sec. Insert a maxi column to a manifold according to manufacturer's instruction.
8. Transfer 20 mL of the sample to a DNA Maxi column, apply vacuum to allow the lysate pass through the column. Process the remaining lysate till all lysate pass through the column. *Note: If the flow through gets too slow, spin the column at 5000g in a 50 mL conical tube for 5 minutes.*
9. Add **10 mL DNA Wash Buffer** to the column and allow the liquid pass through the column.
10. Add **10 mL DNA Wash Buffer** to the column and allow the liquid pass through the column.
11. Turn off the vacuum and decant the flow-through liquid inside the manifold. Connect the manifold back to the vacuum. Turn on the vacuum to dry the column for 15 min.
12. Optional: Turn off the vacuum, transfer the column to a 50 mL conical tube and centrifuge at 5000g for 5 minutes. *Note: Wipe off any ethanol residual inside the column with kimwipe if any. Step 11 and 12 remove residual ethanol for optimized elution in the next step. Swing-bucket type rotor is preferred for centrifugation.*
13. Transfer the column to a clean 50 mL tube and add 1**.5-2.0 mL Endofree Elution Buffer** to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 5,000g for 5 min.
14. Add the eluted DNA back to the column and centrifuge at 5,000 x g for 5 min.

VIII. Centrifuge Protocol:

1. From step 5, spin the lysate at 10,000 rpm for 20 min. Transfer the clear lysate to a clean 50 mL tube. Transfer the clear lysate to a clean 50 mL tube
2. Add 10 mL Buffer RET and 10 mL ethanol, mix well by vortexing for 5 seconds.
3. Apply 20 mL of sample to a DNA Maxi column with the collection tube, spin at 5000 rpm for 5 min. Discard the flow through and put the column back to the collection tube. Repeat loading the remaining sample.
4. Add 10 mL DNA Wash Buffer and spin at 5000 rpm for 5 min. Discard the flow through and put the column back into the collection tube. Repeat once.
5. Spin the maxi column at 5000 rpm for 10 min to dry the column
6. Transfer the column to a clean 50 mL tube and add 1.5-2 mL of Elution Buffer. Spin at 5000 rpm for 5 min to elute the DNA.
7. Optional: Add the eluted DNA back to the column for a second elution.

IX. 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 vortexing 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 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.