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ToxOut™ Express Endofree Plasmid Maxi Kit

(Catalog # K1336-2, -10, -25; Store at multiple temperatures)

I. Introduction:

BioVision's ToxOut™ Express Endofree Plasmid Maxi Kit is designed for fast and efficient purification of plasmid DNA from 150-200 mL of *E. coli* culture. Our proprietary Buffer RET system allows the highly efficient binding of DNA to our ezBind matrix while endotoxin, proteins and other contaminants are removed under certain optimal conditions. DNA is eluted in sterile water or elution buffer. The purified DNA is ion exchange resin residues free which enables the highest performance in downstream applications such as transfection, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations. 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 volumes of cultures and the buffers need to be scaled up 3-5 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:

	K1336-2	K1336-10	K1336-25	
Components	**2 preparations**	**10 preparations**	**25 preparations**	**Part Number**
DNA Columns	2	10	25	K1336-XX-1
Filter Syringe (60 mL)	2	10	25	K1336-XX-2
2.0 mL Centrifuge Tubes	4	20	50	K1336-XX-3
Plastic Wrench	1	1	1	K1336-XX-4
Buffer A1	25 mL	110 mL	220 mL	K1336-XX-5
Buffer B1	25 mL	110 mL	220 mL	K1336-XX-6
Buffer N3	12 mL	60 mL	120 mL	K1336-XX-7
Buffer RET	25 mL	110 mL	220 mL	K1336-XX-8
DNA Washing Buffer	6 mL	50 mL	2 X 50 mL	K1336-XX-9
Elution Buffer	5 mL	25 mL	60 mL	K1336-XX-10
RNase A	100 µL	450 µL	900 µL	K1336-XX-11

***Add 24 mL (K1336-2) or 200 mL (K1336-10, K1336-25) of 100% ethanol to each DNA Wash Buffer bottle before use**

IV. User Supplied Reagents and Equipment:

- High speed centrifuge
- 100% ethanol
- 50 mL conical tubes

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Except Buffer A1 (once RNase A is added), which is stored at 4°C, all other components are stored at RT (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: 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. Store at 4°C.
- Buffer B1 precipitates below RT. It is critical to warm up the buffer at 37°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure a centrifuge capable of 13,000 rpm is available. Especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- Buffer RET and N3 contains acetic acid, wear gloves and protective eyewear when handling.
- Carry out all centrifugations at RT.
- Add 24 mL (K1336-2) or 200 mL (K1336-10, K1336-25) of 100% ethanol to each DNA Wash Buffer bottle before use.

VII. Endofree express 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 3-5 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** 150-200 mL LB containing appropriate antibiotic with 100 µl fresh starter bacterial culture. Grow at 37°C for 14-16 h 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). Warning: Do not use more than 200 ml culture. Need to scale up buffers if processing more than 200 mL 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. *Note: Complete removal of residue medium is critical for bacteria lysis in the next step.*

3. Add 10 mL **Buffer A1** and completely resuspend bacterial pellet by vortexing or pipetting. Ensure that RNase A has been added into Buffer A1 before use. *Note: Complete resuspension is critical for optimal yield. Note: if RT is below 25°C, warm up the mix solution after adding Buffer ER at 45°C for 5 min.*
4. Add 10 mL **Buffer B1**, mix thoroughly by inverting 10 times with gentle shaking. Incubate for 5 min to obtain a slightly clear lysate. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent. *Attention: Buffer B1 forms precipitates below RT, if solution becomes cloudy, warm up at 37°C to dissolve before use.*
5. Add 4 mL **Buffer N3**, mix completely by inverting the tube 10-15 times. Incubate at RT for 10 min. 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. Pour the lysate into the barrel of the filter syringe and set the syringe in a 50 mL conical tube. Incubate at RT for 10 min. The white precipitates should float to the top. Gently insert the plunger to expel the cleared lysate to the 50 mL tube. Stop when you feel strong resistance, some of the lysate may remain in the flocculent precipitate. *Note: To avoid clogging of the syringe: Use less than 200 mL of overnight culture and mix the lysate well after adding Buffer N3. Spin the lysate down at 5000 rpm for 5 min and transfer the relatively clear lysate to the syringe filter.*
7. Add 10 mL **Buffer RET**. Mix well by sharp hand shaking for 5 times.
8. Pull the plunger out from a **DNA column** and set the column in a 50 mL conical tube. Add 20 mL lysate into the DNA column. Using the plunger, gently expel the lysate through the column. Gently pull out the plunger and process the remaining lysate as described. Discard the flow-through.
9. Detach the end component using the plastic wrench and gently pull the plunger out. Screw the end component back to the plunger and add 20 mL **DNA wash buffer**. Expel the Buffer out with the plunger.
10. Use the plastic wrench (provided) to detach the end component from the maxiprep column and insert it into a **2 mL Centrifuge tube**.
11. Spin the column at 13,000-15,000 rpm (max speed) for 2 min. Decant the flow-through and put the column back in the tube. Spin the column at max speed for 1 min. Transfer the column to a new eppendorf tube.
12. Add 400 µL **Elution Buffer** (pre-warming the Elution Buffer at 60°C increases the yield) to the center of the column and incubate for 1 min at RT. Elute the DNA by centrifugation at 12,000 rpm for 1 min.
13. If higher DNA concentration is desired, add the eluted DNA from step 12 back to the column and spin at 12,000 rpm to elute the DNA. The first elution normally yields about 60-70% of the DNA and the second elution yields another 20-30% of the DNA.

VIII. Related Products:

Product Name	Cat. No.
ToxOut™ Endofree Plasmid Mini Kit	K1326-50
ToxOut™ Endofree Plasmid Mini Kit	K1326-250
ToxOut™ Endofree Plasmid Mini Kit II	K1327-50
ToxOut™ Endofree Plasmid Mini Kit II	K1327-250
ToxOut™ Endofree Plasmid Midi Kit	K1328-2
ToxOut™ Endofree Plasmid Midi Kit	K1328-10
ToxOut™ Endofree Plasmid Midi Kit	K1328-25
ToxOut™ Endofree ezFilter Plasmid Midi Kit	K1329-2
ToxOut™ Endofree ezFilter Plasmid Midi Kit	K1329-10
ToxOut™ Endofree ezFilter Plasmid Midi Kit	K1329-25
ToxOut™ Endofree Plasmid Maxi Kit	K1330-2
ToxOut™ Endofree Plasmid Maxi Kit	K1330-10
ToxOut™ Endofree Plasmid Maxi Kit	K1330-25
ToxOut™ Endofree ezfilter Plasmid Maxi Kit	K1331-2
ToxOut™ Endofree ezfilter Plasmid Maxi Kit	K1331-10
ToxOut™ Endofree ezfilter Plasmid Maxi Kit	K1331-25
ToxOut™ Endofree Plasmid Mega Kit (3 mg)	K1332-1
ToxOut™ Endofree Plasmid Mega Kit (3 mg)	K1332-2
ToxOut™ Endofree Plasmid Mega Kit (3 mg)	K1332-10
ToxOut™ Endofree Plasmid Mega Kit (6 mg)	K1333-1
ToxOut™ Endofree Plasmid Mega Kit (6 mg)	K1333-2
ToxOut™ Endofree Plasmid Mega Kit (6 mg)	K1333-10
ToxOut™ Endofree Plasmid Mega Kit (10 mg)	K1334-1
ToxOut™ Endofree Plasmid Mega Kit (10 mg)	K1334-2
ToxOut™ Endofree Plasmid Mega Kit (10 mg)	K1334-10
ToxOut™ Express Endofree Plasmid Midi Kit	K1335-2
ToxOut™ Express Endofree Plasmid Midi Kit	K1335-10
ToxOut™ Express Endofree Plasmid Midi Kit	K1335-25
ToxOut™ Express Endofree Plasmid Maxi Kit	K1336-2
ToxOut™ Express Endofree Plasmid Maxi Kit	K1336-10
ToxOut™ Express Endofree Plasmid Maxi Kit	K1336-25

IX. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low Yield	• Poor Cell lysis. • Bacterial culture. overgrown or not fresh. • Low copy number plasmid.	• Resuspend the pellet thoroughly by vortexing and pipetting prior to adding Buffer B1. • Make fresh Buffer B1 if the cap was 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 silica membrane before elute the plasmid DNA. Re-centrifuge again if necessary.

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