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BioVision
BioVision Incorporated

ToxOut™ Endofree Plasmid Mini Kit II

(Cat# K1327-50, -250; Store at RT)

I. Introduction:

BioVision's ToxOut™ Endofree Plasmid Mini Kit II is designed for fast and efficient purification of plasmid DNA from 3-12 mL of *E. coli* culture. The mini column II has a DNA binding capacity of 80 µg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.
Plasmid isolated with traditional protocol normally contains high level of endotoxins (lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The endofree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per µg of plasmid DNA. The Toxout™ Endofree Plasmid Mini Kit II provides an efficient endotoxin removal step into the traditional purification procedure to produce transfection grade plasmid DNA. The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid.

II. Sample Type: For fast and efficient purification of endotoxin free plasmid DNA from 3-12 mL of *E. coli* culture.

III. Kit Contents:

Components	K1327-50 50 Preparations	K1327-250 250 Preparations	Part Number
ezBind Columns	50	250	K1327-XX-1
Buffer A1*	25 mL	130 mL	K1327-XX-2
Buffer B1	25 mL	130 mL	K1327-XX-3
Buffer N3	10 mL	65 mL	K1327-XX-4
Buffer KB	30 mL	150 mL	K1327-XX-5
Buffer RET	30 mL	130 mL	K1327-XX-6
DNA Wash Buffer**	12 mL **(add 48 ml 96-100% ethanol)**	50 mL **(add 200 ml 96-100% ethanol)**	K1327-XX-7
Endofree Elution Buffer	15 mL	30 mL	K1327-XX-8
RNase A	110 µl	13 mg	K1327-XX-9

Add the RNase A solution to buffer A1 and mix well before use. **Add 48 mL (K1327-50) or 200 mL (K1327-250) of 96-100% ethanol to each DNA Wash Buffer bottle before use.

IV. User Supplied Reagents and Equipment:

- High speed microcentrifuge; 30 ml high speed centrifuge tubes
- 96-100% ethanol; 2.0 mL microcentrifuge tubes

V. Shipment and Storage:

All the reagents are shipped and stored 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: It is stable for half a year under room temperature. Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use.
- Add 48 mL (K1327-50) or 200 mL (K1327-250) of 96-100% ethanol to each DNA Wash Buffer bottle before use.
- Buffer B1 precipitates below RT. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- Buffer N3 may form precipitates below 10°C, warm up at 37°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use. Ensure the availability of centrifuge capable of 13,000 rpm.
- Carry out all centrifugations at room temperature.

VII. Endofree Plasmid Miniprep Protocol:

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 **5-10 mL** LB containing appropriate antibiotic with a single colony from a freshly streaked selective plate. Grow at 37°C for 14-16 hr with vigorous shaking. *Note: Do not use a streaked plate that has been stored at 4°C. Note: Do not inoculate culture directly with glycerol stock. Note: This protocol is optimized for E. coli strain cultured in LB medium. When using TB or 2XYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers need to be scaled up if over amount of cultures are being processed.*
2. Harvest the bacterial culture by centrifugation for 1 min at 12,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely. *Note: Residue medium will cause, • Poor cell lysis and thus lower DNA yield. • Loose pellet after centrifugation.*
3. Add **450 µL** Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting. (Complete resuspension is critical for bacterial lysis and lysate neutralization). Ensure RNase A has been added in Buffer A1 before use.
4. Add **450 µL** Buffer B1, mix gently by inverting 10 times (do not vortex) and incubate at room temperature for 5-10 min. ***Note: Do not incubate for more than 5 min.***
5. Add **100 µL** Buffer N3, mix completely by inverting/shaking the vial for 3 times. ***Note: It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mixing is required to completely neutralize the solution.***
6. Centrifuge the lysate at 13,000 rpm for 10 min at room temperature. ***Note: If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more min and then transfer the clear lysate to DNA column.***
7. Carefully transfer the clear lysate (no more than 800 µL) to a clean 2.0 mL tube and add **550 µL** of Buffer RET, and **450 µL** of 100% ethanol. Mix well by vortexing for 2 sec.

8. Transfer **700 µL** of the lysate/ethanol mixture to a DNA spin column and centrifuge at 13,000 rpm for 20 sec. Discard the flow-through liquid and transfer the remaining lysate/ethanol mixture to the column. Centrifuge at 13,000 rpm for 30 sec and discard the flow-through, put the column back to the collection tube.
9. Add **500 µL** Buffer KB into the spin column, centrifuge at 13,000 rpm for 1 min. Remove the spin column from the tube and discard the flow through. Put the column back to the collection tube. ***Note: This step is important to remove residual protein contaminations especially for endA+ strains and be highly recommended for high quality plasmid DNA.***
10. Add **500 µL** DNA Wash Buffer and centrifuge at 13,000 rpm for 20 sec. Discard the flow-through liquid and insert the column, with the lid open, back to the collection tube. Repeat step "10".
11. Centrifuge the empty column, with the lid open, at 13,000 rpm for 1 min to remove the residual ethanol. *Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.*
12. Transfer the column to a 1.5 mL tube and add **50-100 µL** of Endofree Elution Buffer Incubate for 1 min at room temperature and centrifuge at 13,000 rpm for 1 min to elute DNA. Reload the eluate into the column (use the same 1.5 mL tube) and incubate for 1 min, centrifuge at 13,000 rpm for 1 min to elute DNA. *Note: The eluted DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.*

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 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. Recentrifuge or vacuum again if necessary.

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