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BioVision
BioVision Incorporated

ToxOut™ Endofree Plasmid Mega10 Kit

(Cat# K1334-1, -2, -10; Store at RT)

I. Introduction:

BioVision's ToxOut™ Endofree Plasmid Mega10 Kit is designed for fast and efficient purification of plasmid DNA from 1500 mL of *E. coli* culture. The maxi column has a DNA binding capacity of 10-12 mg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection. The endofree system uses a formulated buffer to remove the endotoxin during or post plasmid DNA purification. The endotoxin level after purification is < 0.2 EU/µg 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 1500 mL of *E. coli* culture.

III. Kit Contents:

	K1334-1	K1334-2	K1334-10	
Components	**1 preparation**	**2 preparations**	**10 preparations**	**Part Number**
DNA Unit	1	2	10	K1334-XX-1
Filter Unit	1	2	10	K1334-XX-2
Replacement Cup	2	4	20	K1334-XX-3
Buffer A1	110 mL	210 mL	1050 mL	K1334-XX-4
Buffer B1	110 mL	210 mL	1050 mL	K1334-XX-5
Buffer N3	40 mL	110 mL	550 mL	K1334-XX-6
Buffer RET	110 mL	210 mL	1050 mL	K1334-XX-7
Endofree Elution Buffer	30 mL	60 mL	270 mL	K1334-XX-8
RNase A	450 µL	600 µL	3000 µL	K1334-XX-9

IV. User Supplied Reagents and Equipment:

- 70% ethanol and 100% ethanol and isopropanol
- Vacuum system
- 250 mL or 500 mL or 1,000 mL bottle or equivalent pyrex glass bottles
- 50 mL conical tubes

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: It is stable for more than half a year when stored at room temperature. Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.
- Buffer B1 precipitates below room temperature. 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 the availability of centrifuge and vacuum manifold, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by vacuum.

VII. <u>Endofree ezFilter Megaprep10 Purification 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.

1. Inoculate 800-1000 mL LB containing appropriate antibiotic with 500 µL fresh starter culture. Grow at 37°C for 14-16 hr with vigorous shaking. Harvest 500 mL overnight bacterial cells by centrifugation at 5000g for 10 mins at room temperature. Decant or aspirate medium and discard. *Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 2 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 hr with vigorous shaking (~250 rpm).*
2. Resuspend the bacterial pellet in 100 mL Buffer A1 (Add RNase A into Buffer A1 before use). Pipet or vortex till the bacterial pellet dispersed thoroughly (Complete resuspension is critical for optimal yields).
3. Add 100 mL Buffer B1, mix thoroughly by inverting 10 times with mild shaking. Incubate for 10 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 precipitation below room temperature, if solution becomes cloudy, warm up at 37°C to dissolve before use.*
4. Add 50 mL Buffer N3 and mix immediately by inverting vigorously 5-10 times till a flocculent white precipitate forms. Note: It is critical to mix the lysate well, if the mixture still appears conglobated, brownish or viscous, more mix is required to completely neutralize the solution.
5. Spin the sample at 5,000 rpm for 10 min.

6. Attach the 2-layer filter unit to a sterile 500 mL or 1000 mL standard bottle and screw tight. Connect the unit to a pump-driven vacuum system.
7. Transfer the relatively clear lysate from step 5 (use a 50 mL serological pipet) to the filter unit. Stand by for 2 minute and turn on the vacuum. Load the remaining lysate till all lysate passes through the filter unit.
   Note 1: *Use a 50 mL serological pipet to transfer the relatively clear to the filter unit. Normally around 220 mL lysate can be filtered through the filter unit within 10 min.* ***Note 2:*** *If the flow through gets too slow, turn off the vacuum and wait for 1 min. Carefully detach the upper filter cup and replace it with a replacement cup. Pour the lysate from the original cup to the replacement cup. Turn on the vacuum and filter the rest of the lysate.*
8. When most of the lysate has been filtered through the unit, turn off the vacuum, wait for 1 min, detach the unit and discard the filter unit. ***Note:*** *The DNA is in the collecting bottle.*
9. Connect a DNA unit to a clean 500 mL bottle and screw tight. Connect the DNA binding unit to the vacuum with the vacuum off. Add 100 mL Buffer RET to the sample. Mix well by inverting vigorously. Pour lysate/ethanol mixture to the DNA binding unit to the top level and turn on the vacuum.
10. Transfer the remaining lysate/ethanol mixture into the DNA binding unit. When all the lysate passes through the DNA binding unit, vacuum for another 2 min.
11. Add 80 mL 70% ethanol evenly to the DNA membrane and allow the liquid to pass through the DNA membrane.
12. Add 80 mL 100% ethanol evenly to the DNA membrane and allow the liquid to pass through the DNA membrane. Turn off the vacuum, wait for 1 min, and discard the flow through liquid from the collection bottle. Reconnect the DNA unit to the collection bottle.
13. Turn on the vacuum for 20 min at maximum force (It is critical to dry the residual ethanol for optimal yield).
14. Turn off the vacuum, wait for 1 min, and replace the 500 mL or 1,000 mL bottle with a sterile 50 mL conical tube, screw tight.
15. Add 10-15 mL Endofree Elution Buffer evenly to the membrane and incubate at room temperature for 2 min. Turn on vacuum to elute DNA. Typically, 5-8 mL of DNA containing solution can be collected. Turn off the vacuum. Add the eluted DNA back to the DNA unit for another elution. ***Note:*** *The first elution normally yields 60-70% of the DNA while the second elution recovers another 20% of the DNA bound to the membrane.*
16. The DNA is ready for downstream application such as transfection of difficult- to-transfection cells and primary cells.

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 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 the culture volume to 2X of original volume. Increase the volume of buffer A1, B1, N3 according to instructions.
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.