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BioVision
BioVision Incorporated

ToxOut™ Endofree Plasmid Mega6 Kit

(Catalog # K1333-1, -2, -10; Store at multiple temperatures)

I. Introduction:

BioVision's ToxOut™ Endofree Plasmid Mega6 Kit is designed for fast and efficient purification of plasmid DNA from 1000 mL of *E. coli* culture. The maxi column has a DNA binding capacity of 6-7 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 1000 mL of *E. coli* culture.

III. Kit Contents:

Components	K1333-1 1 preparation	K1333-2 2 preparations	K1333-10 10 preparations	Part Number
DNA Unit	1	2	10	K1333-XX-1
Filter Unit	1	2	10	K1333-XX-2
Replacement Cup	1	4	20	K1333-XX-3
Buffer A1	60 mL	130 mL	2 X 320 mL	K1333-XX-4
Buffer ER	3 mL	6.5 mL	32 mL	K1333-XX-5
Buffer B1	60 mL	130 mL	2 X 320 mL	K1333-XX-6
Buffer D1	6 mL	13 mL	64 mL	K1333-XX-7
Buffer N3	15 mL	35 mL	160 mL	K1333-XX-8
Buffer RET	130 mL	260 mL	3 X 420 mL	K1333-XX-9
Endofree Elution Buffer	30 mL	60 mL	270 mL	K1333-XX-10
RNase A (20 mg/mL)	6 mg (300 µL)	12.5 mg (625 µL)	2 X 32 mg (2 X 1.6 mL)	K1333-XX-11

IV. User Supplied Reagents and Equipment:

- 70% ethanol and 100% ethanol
- Pump-driven vacuum system, 1,000 mL bottle or equivalent pyrex glass bottles
- 50 mL conical tubes
- High speed centrifuge tubes for endotoxin removal if desired

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 ER should be stored 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.
- 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.
- 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. Endofree ezFilter Megaprep6 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 2-3 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. *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). The buffer volumes need to be scaled up if processing over 1000 mL of culture.*
2. Harvest 800-1000 mL overnight bacterial cells by centrifugation at 5,000g for 10 min at room temperature. Decant or aspirate medium and discard. Note: Remove the residual medium completely for optimal cell lysis and neutralization.
3. Resuspend the bacterial pellet in 60 mL Buffer A1 (Add RNase A to Buffer A1 before use). Pipet or vortex till the bacterial pellet dispersed thoroughly (Complete resuspension is critical for optimal yields). Then add 3 mL Buffer ER into the suspended bacterial culture. Mix well by inverting 5-10 times.

4. Add 54 mL Buffer B1. Mix gently but thoroughly by inverting 10 times and incubate at room temperature for 5 min to obtain a cleared lysate. Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage. Avoid vigorous mixing as this will shear the genomic DNA. Then add 6 mL Buffer D1, mix gently and incubating for another 5 min
5. Add 15 mL Buffer N3 and mix immediately by inverting 5 times till a flocculent white precipitate forms. Vortex the lysate for 5 sec. *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.*
6. Attach the 2-layer filter unit to a sterile 500 mL or 1000 mL standard bottle or equivalent pyrex glass bottle and screw tight. Connect the unit to a pump-driven vacuum system.
7. Transfer the clear lysate from the bottom of the mixture (use a 50 mL serological pipet) to the filter unit. Stand by for 5 min and turn on the vacuum with low vacuum force and increase to maximum vacuum force after 5 min. *Note 1: Low vacuum force prevents clogging of the filter membranes. Note 2: Use a 50 mL serological pipet to transfer the relatively clear lysate from the bottom of the lysate bottle to the filter unit. This will speed up the flow rate of the filter unit. Normally around 120 mL lysate can be filtered through the filter unit within 20-30 min. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through. Note 3: 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 the 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 upper filter cup including the rubber rings. *Note: The DNA is in the collection bottle.*
9. Connect the DNA unit to a 500 mL or 1,000 mL standard bottle and screw tight. Connect the DNA unit to the vacuum with the vacuum off. Add 1 volume of Buffer RET (For example, 120 mL of Buffer RET to 120 mL of clear lysate), and add 60 mL 100% ethanol to the lysate bottle. Mix well by sharp hand shaking 3-5 times and immediately pour half of the lysate/ethanol mixture to the DNA unit and turn on the vacuum.
10. Pour the rest of the lysate/ethanol mixture into the DNA unit. When all the lysate passes through the DNA unit, vacuum for 1 min.
11. Wash the DNA membrane with 50 mL 70% ethanol and vacuum for 1 min at maximum force. Wash the DNA membrane with another 50 mL 70% ethanol and vacuum for 1 min at maximum force.
12. Add 60 mL 100% ethanol evenly to the DNA membrane and vacuum for 1 min. Turn off the vacuum, wait for 1 min, and discard the liquid waste in the bottle. Reconnect the bottle to the DNA binding unit. Turn on the vacuum for 20 min at maximum force (It is critical to dry the residual ethanol for optimal yield).
13. Turn off the vacuum, wait for 1 min, and replace the 500 mL or 1,000 mL standard bottle with a sterile 50 mL conical tube, screw tight.
14. Add 10 mL Endofree Elution Buffer evenly to the membrane and incubate for 2 min. Turn on vacuum to elute DNA. Typically, 3~5 mL of solution can be collected. This is the 1st elution.
15. Turn off the vacuum and replace the 50 mL conical tube with another sterile 50 mL conical tube, screw tight. Add 8 mL Endofree Elution Buffer and incubate for 1 min. Turn on the vacuum and collect the 2nd elution, typically 3 ~6 mL of solution can be collected. *Note: The DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection. Note: Two elution give rise to maximum DNA yield. For maximum yield and higher concentration, pool the elution together, add 0.1 volume 3M Potassium Acetate or Sodium acetate (pH 5.2) and 0.7 volume isopropanol. Centrifuge at top speed for 10 min. Discard supernatant. Wash the DNA with 1000 µL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 10-20 minutes in a tissue culture hood. Resuspend the DNA in Endofree Elution Buffer. Note: Use less Endofree Elution Buffer if high concentration is desired.*

DNA concentration (µg/mL) = $OD_{260\ nm}$ X 50 X dilution factor

VIII. Purification of Low-Copy-Number Plasmid/Cosmid: The yield of low copy number plasmid is normally around 0.1-1 µg /mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

1. Culture volume: Use 2X volumes of the high copy number culture.
2. Use 2X volume of the Buffer A1, Buffer B1, Buffer N3 and 100% ethanol.
3. Use same volume of Wash Buffer (70% ethanol and 100% ethanol) and Endofree Elution Buffer.

IX. 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

X. 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 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.