Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

ToxOut™ Endofree Plasmid Mega3 Kit

Rev 12/19

(Catalog # K1332-1, -2, -10; Store at multiple temperatures)

I. Introduction:

BioVision's ToxOut™ Endofree Plasmid Mega3 Kit is designed for fast and efficient purification of plasmid DNA from 500 mL of *E. coli* culture. Our proprietary DNA binding systems allow highly efficient binding of DNA to our ezBind™ matrix while proteins and other contaminants are removed. Nucleic acids are easily eluted with sterile water or TE buffer. Our proprietary endotoxin removal buffer, Buffer RET, is designed to remove endotoxin by a single washing step without tedious phase partitioning steps. The purified plasmid DNA has endotoxin levels less than 1 EU per µg of DNA. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

II. Sample Type: *E. coli* culture.

III. Kit Contents:

Components	K1332-1 (1 prep)	K1332-2 (2 preps)	K1332-10 (10 preps)	Part Number
Buffer A1	35 ml	70 ml	650 ml	K1332-XX-1
Buffer B1	35 ml	70 ml	650 ml	K1332-XX-2
Buffer C1	15 ml	25 ml	700 ml	K1332-XX-3
Buffer RET	35 ml	70 ml	650 ml	K1332-XX-4
DNA Wash Buffer*	50 ml	2 x 50 ml	2 x 200 ml	K1332-XX-5
DNA Unit	1	2	10	K1332-XX-6
Filter Unit	1	2	10	K1332-XX-7
Filter Unit Replacement Cup	2	4	20	K1332-XX-8
Endofree Elution Buffer	30 ml	50 ml	250 ml	K1332-XX-9
RNase A	120 µl	200 µl	2 ml	K1332-XX-10

**DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 200 ml (K1332-1 and K1332-2) or 800 ml (K1332-10) to DNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.*

IV. User Supplied Reagents and Equipment:

- 100% ethanol
- Pump-driven vacuum system
- 250 ml or 500 mL bottle (Corning# 430282) or 1,000 mL bottle (#430518) or equivalent.
- 50 ml conical tubes
- High speed centrifuge

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Buffer A1 (once RNase A is added), should be stored at 4°C, all other components are stored at RT. 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 all of the RNase A solution to Buffer A1 and mix well before use.
- DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 200 ml (K1332-1 and K1332-2) or 800 ml (K1332-10) to 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.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure that centrifuge capable of spinning at 13, 000 rpm is available.

VII. Plasmid ezFilter Megaprep 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. Culture Medium: This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) to a density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2xYT are used, make sure the cell density does not exceed 3.0 (OD_{600}). A high ratio of cell density over lysis buffers result in low DNA yield and purity. For higher cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, B1 and C1.

1. Inoculate **500 ml LB** containing appropriate antibiotic with **500 µl fresh starter 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 2 mL LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 hr with vigorous shaking (~250 rpm).*
2. Harvest **500 ml overnight bacterial cells** by centrifugation at 5,000 g for 10 min at RT. Decant or aspirate medium and discard.
3. Resuspend the bacterial pellet in **30 ml Buffer A1** (Add RNase A to Buffer A1 before use). Pipet or vortex till the bacterial pellet disperses thoroughly (Complete resuspension is critical for optimal yields).
4. Add **30 ml Buffer B1**. Mix thoroughly by inverting 10 times with mild shaking. Incubate at RT for 5-10 min to obtain a clear lysate. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent. Buffer B1 forms precipitates below RT, if solution becomes cloudy, warm up at 37°C to dissolve before use.

5. Add **10 ml Buffer C1** and mix immediately by inverting 5 times till a flocculent white precipitate forms. Mix the lysate well by sharp shaking 5 times. *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. Incubate the mixture at RT for 10 min.
7. Attach the **2-layer filter unit** to a **sterile** 500 ml or 1000 ml standard bottle (Corning# 430518 or 430282 or equivalent) and screw tight. Connect the unit to a pump-driven vacuum system.
8. **Spin** the sample at 5000 rpm for 5 min. Transfer the clear lysate of the mixture (use a 50 ml serological pipet) to the filter unit. Stand by for 2 min and turn on the vacuum. *Note 1:* Start with Low vacuum force and increase to high force. This prevents clogging of the filter membranes. *Note 2: Use a 50 ml serological pipet to transfer the relatively clear lysate to the filter unit. This will speed up the flow rate of the filter unit. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through. Normally around 150 ml lysate can be filtered through the filter unit within 10 min. 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.*
9. 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 solution in the collection bottle.*
10. Connect the **DNA unit** to a 500 ml clean bottle and screw tight. Connect the DNA binding unit to the vacuum with the vacuum off. Add 30 ml of **Buffer RET** and 30 ml 100% ethanol to the lysate bottle. Mix the sample well by vortexing for 10 sec and pour the lysate mixture to the DNA binding unit and turn on the vacuum.
11. When all the lysate passes through the DNA binding unit, vacuum for another 2 min.
12. Add **50 ml DNA Wash Buffer** evenly to the DNA membrane and vacuum for 5 min to allow the buffer pass through the membrane.
13. Add **50 ml DNA Wash buffer** to the DNA membrane and vacuum for 5 min. Turn off the vacuum, wait for 1 min and discard the liquid waste in the bottle. Reconnect the bottle to DNA binding unit.
14. Turn on the vacuum for 30 min at maximum force (It is critical to dry the residual ethanol for optimal yield).
15. 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.
16. Add **15 ml Endofree Elution Buffer** evenly to the membrane and incubate for 5 min at RT. Turn on vacuum to elute DNA. Typically, 8-12 ml of DNA containing solution can be collected. Turn off the vacuum. Note: If high DNA concentration is desired, add the eluted DNA back to the DNA unit for a second elution.
17. Add another **10 ml Elution Buffer** evenly to the membrane and incubate for 2 min. Turn on vacuum to elute DNA.

VIII. Related Products:

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

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: 0.2N 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 (up to 10 mL for Minipreps, 100mL for Midipreps, 200 mL for Maxipreps and 3L for Megapreps). Increase the volume of buffer A1, B1, C1 and ethanol proportionally with the ratio of 1:1:1.2:1.2. .
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.

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