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# ToxOut™ Endofree ezFilter Plasmid Midi Kit

**(Catalog # K1329-2, -10, -25; Store at multiple temperatures)**

## I. Introduction:

BioVision's ToxOut™ Endofree ezFilter Plasmid Midi Kit is designed for fast and efficient purification of plasmid DNA from 80-100 mL of *E. coli* culture. The midi column has a DNA binding capacity of 500 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. 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, with endotoxin level less than 0.1 EU per µg of DNA, is ready for transfection of endotoxin sensitive cell lines and microinjections. 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.

## II. Sample Type:

For fast and efficient purification of endotoxin free plasmid DNA from 80-100 mL of *E. coli* culture.

## III. Kit Contents:

| | K1329-2 | K1329-10 | K1329-25 | Part Number |
|---|---|---|---|---|
| **Components** | **2 preparations** | **10 preparations** | **25 preparations** | |
| Midi Columns | 2 | 10 | 25 | K1329-XX-1 |
| Filter Syringe | 2 | 10 | 25 | K1329-XX-2 |
| Buffer A1 | 12 mL | 60 mL | 135 mL | K1329-XX-3 |
| Buffer B1 | 12 mL | 60 mL | 135 mL | K1329-XX-4 |
| Buffer N3 | 6 mL | 30 mL | 70 mL | K1329-XX-5 |
| Buffer RET | 12 mL | 60 mL | 135 mL | K1329-XX-6 |
| RNase A | 50 µL | 200 µL | 420 µL | K1329-XX-7 |
| DNA Wash Buffer | 12 mL | 50 mL | 50 mL | K1329-XX-8 |
| Endofree Elution Buffer | 2 mL | 15 mL | 60 mL | K1329-XX-9 |

*DNA Wash buffer must be diluted with 100% ethanol before starting. Add 48 mL (K1329-2) or 200 mL (K1329-10 and K1329-25) of 100% ethanol to each DNA Wash Buffer bottle before use.*

## IV. User Supplied Reagents and Equipment:

- Centrifuge with swinging bucket rotor and centrifuge tubes
- 70% ethanol and 100% ethanol
- 15 mL and 50 mL Conical tubes
- Vacuum manifold
- Pump driven vacuum system capable of generating 200-600 mPa

## V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Except Buffer A1 (once RNase A is added), 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: 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 50°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- DNA Wash buffer must be diluted with 100% ethanol before starting. Add 48 mL (K1329-2) or 200 mL (K1329-10 and K1329-25) of 100% ethanol to each DNA Wash Buffer bottle before use.
- Ensure the availability of centrifuge capable of 5, 000 x g.
- Carry out all centrifugation steps at RT.

## VII. Endofree ezFilter Midiprep 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.

Host strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10, DH5a, and C600 yield high-quality plasmid DNA. *endA*+ strains such as JM101, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend transforming plasmid to an *endA*- strain if the yield is not satisfactory.

1. Inoculate 80-100 mL LB containing appropriate antibiotic with 100 µL fresh starter culture. Inoculate 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. Grow at 37°C for 8 hr with vigorous shaking (~250 rpm) and use the culture as starter culture.*

2. Harvest the bacteria by centrifugation at 5,000g for 10 min at RT. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium completely.
3. Add 5 mL Buffer A1 (Add RNase A to Buffer A1 before use) and completely resuspend bacterial pellet by vortexing or pipetting. Transfer the lysate to a 15 mL conical tube
4. Add 5 mL Buffer B1, mix thoroughly by inverting 10 times with slight shaking. Incubate for 5 min to obtain a slightly clear lysate. Complete lysis is critical for DNA yield.
   *Note: Buffer B1 forms precipitates below RT. Dissolve the precipitates at 37°C before use.*
5. Add 2 mL Buffer N3, mix completely by inverting 10 times and sharp shaking for 5 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. Place the barrel of the syringe in a clean 50 mL conical tube (not supplied) set in a rack. Transfer the lysate into the barrel of the filter syringe and incubate at RT for 10 minutes. Gently insert the plunger to expel the cleared lysate into the tube.
   Note: The cleared lysate should be clear and free of cloudy precipitates. If it looks turbid or cloudy, add 0.5 mL of Buffer N3, mix well and filter through another syringe filter. Alternatively, the sample can be centrifuged at 5, 000 rpm for 5 min, transfer the clear supernatant to a clean 15 mL conical tube.
7. Add 5 mL Buffer RET and 5 mL of 100% ethanol. Mix well by vortexing for 5 sec. Insert a midi column to a manifold according to manufacturer's instruction.
8. Transfer 20 mL of sample to the DNA midi column, apply vacuum to allow the lysate pass through the column. Process the remaining lysate till all lysate passes through the column.
   Note: If the flow through gets too slow, spin the column at 5,000 x g in a 15 mL conical tube for 2 minutes.
9. Add 10 mL DNA Wash Buffer to the column and allow the liquid to pass through the column. Turn off the vacuum and decant the flow-through liquid inside the manifold. Connect the manifold back to the vacuum. Turn on the vacuum to dry the column for 15 min.
   Note: Alternatively, the column can be dried by spinning at 5,000 rpm for 10 minutes. It is critical to dry the column completely for optimized DNA yield and purity.
10. Transfer the column to a clean 50 mL conical tube and add 0.5 -1 mL endofree Elution Buffer to the center of the column and incubate for 2 min at RT. Elute the DNA by centrifugation at 5,000 x g for 5 min.
11. Add the eluted DNA back to the column and centrifuge at 5,000 x g for 5 min.
    Note: The first elution yields approximately 70% of the DNA. The second elution yields another 20% of the DNA. The midiprep column has high DNA binding capacity (500 µg).

**Note:** The DNA is ready for downstream applications such as transfection of endotoxin sensitive cell lines and microinjections.

## 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 |

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| 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.<br>• Bacterial culture overgrown or not fresh.<br>• Low copy number plasmid. | • Resuspend pellet thoroughly by votexing and pipetting prior to adding Buffer B1.<br>• Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N NaOH and 1% SDS).<br>• Grow bacteria for 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.<br>• Increase culture volume (upto 100 ml for Midipreps).. |
| **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 eluting the plasmid DNA. Re-centrifuge again if necessary. |

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