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

Plasmid ezFilter Midi Kit I, Centrifuge

rev 07/19

(Catalog # K1316-2, -10, -25; Store at multiple temperatures)

I. Introduction:

BioVision's Plasmid ezfilter Midi Kit I is designed for fast and efficient purification of plasmid DNA from 15-50 mL of *E. coli* culture. The midi column has a plasmid DNA binding capacity of 250 µg. The yield from 50 mL culture is typically around 150 to 250 µg. The purified DNA is ready for high performance of downstream applications such as transfection of robust cells such as HEK293, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations. Unlike other kits in the markets, our patented plasmid purification kit has no chaotropic salts in the buffers, the purified DNA is guanidine/anion exchange resin residues free which enable the high performance of downstream applications.

II. Sample Type:

For fast and efficient purification of plasmid DNA from 15-50 mL of *E. coli* culture.

III. Kit Contents:

	K1316-2	K1316-10	K1316-25	
Components	**2 preparations**	**10 preparations**	**25 preparations**	**Part Number**
ezBind Columns	2	10	25	K1316-XX-1
Filter Syringe (25 mL)	2	10	25	K1316-XX-2
Buffer A1	6 mL	30 mL	70 mL	K1316-XX-3
Buffer B1	6 mL	30 mL	70 mL	K1316-XX-4
Buffer C1	7 mL	35 mL	85 mL	K1316-XX-5
DNA Wash Buffer*	5 mL	48 mL	90 mL	K1316-XX-6
RNase A (20 mg/mL)	30 µL	150 µL	350 µL	K1316-XX-7
Elution Buffer	5 mL	25 mL	60 mL	K1316-XX-8

Add 20 ml (K1316-2), 192 ml (K1316-10) and 360 ml (K1316-25) 100% ethanol to each **DNA Wash Buffer bottle before use.*

IV. User Supplied Reagents and Equipment:

- 70% ethanol and 100% ethanol.
- High speed centrifuge.
- 30 mL high speed centrifuge tubes.
- 15 mL and 50 mL conical tubes.
- 1.5 mL tubes.
- Isopropanol if precipitate the plasmid DNA.

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Except the Buffer A1 (once RNase A is added), which is 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 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.
- Add 20 ml (K1316-2), 192 ml (K1316-10), and 360 ml (K1316-25) 100% ethanol to each DNA Wash Buffer bottle before use.
- Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation.
- Carry out all centrifugations at RT.

VII. Plasmid Midiprep Spin 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 15-50 mL LB containing appropriate antibiotic with 50 µL fresh starter culture. Incubate 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 8 hr with vigorous shaking (~250 rpm) and then use the culture as starter culture. Note: Do not use more than 50 mL culture or cell mass greater than 150. The buffer volume needs to be scaled up if processing over 100 mL of culture. Note: Do not use a starter culture that has been stored at 4°C. Note: Do not grow starter culture directly from glycerol stock.*
2. Harvest the bacterial 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.
3. Add 2.5 mL Buffer A1 (Add RNase A to Buffer A1 before use) and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).
4. Add 2.5 mL Buffer B1, mix gently but thoroughly by inverting 5 times and incubate for 5 min to obtain a slightly clear lysate. *Note: Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage.*
5. Add 3.0 mL Buffer C1, mix immediately by inverting 5 times and sharp hand shaking for 3 times. *Note: It is critical to mix the solution well. If the mixture still appears conglobated, brownish or viscous, more mix is required to completely neutralize the solution.*

6. **Optional 1:** Transfer the lysate to a high speed centrifuge tube and centrifuge at 14,000g for 10 min at RT. Carefully transfer the clear supernatant into a 15 mL tube (avoid the floating precipitates). *Note: If the rotor is cold, incubate the lysate at RT for 10 min and then perform centrifugation as described.*
   Optional 2: Pour the lysate directly into the barrel of the filter syringe. Insert the syringe to a clean 15 mL tube (not supplied) set in a rack. Allow the cell lysate to sit for 10 min. The white precipitates should float to the top. Hold the filter syringe barrel over the 15 mL tube and gently insert the plunger to expel the cleared lysate to the tube, stop when feel major resistance, some of the lysate may remain in the flocculent precipitate, do not force the residual lysate through the filter.
7. Add 3.0 mL 100% ethanol and mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through to the DNA column immediately.
8. Immediately transfer 6.0 mL the lysate/ethanol mix into a DNA column with a 15 mL collection tube. Centrifuge at >2,500g for 1 min at RT. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube. Repeat step "8" till all the lysate/ethanol mix has been passed through the column.
9. Add 500 µL DNA Wash Buffer (Add ethanol to DNA wash buffer before use) into the spin column, centrifuge at 13,000 rpm (14,000 - 18,000 x g) for 1 min at RT. Remove the spin column from the tube and discard the flow-through.
10. Reinsert the spin column into the collection tube and centrifuge for 1-2 min at 15,000 rpm (Maximum speed).
11. Carefully transfer the column into a sterile clean 15 mL tube and add 0.5-1 mL Elution Buffer or sterile ddH_2O to the center of the column and incubate for 1 min at RT. Elute the DNA by centrifugation at > 2,500g for 5 min.

VIII. 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 and the volume of Buffer A1, B1, N1 as instructed.
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.