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BioVision
BioVision Incorporated

Plasmid ezFilter Maxi Kit

(Cat# K1319, -10, -25; Shipped at RT; Stored at Multiple Temperatures)

I. Introduction:

BioVision's Plasmid ezFilter Maxi Kit is designed for fast and efficient purification of plasmid DNA from 100-250 mL of *E. coli* culture in less than 60 min. The maxi column has a plasmid DNA binding capacity of 1 mg. The purified DNA is ready for high performance of downstream applications such as transfection, RFLP, DNA amplification, and automated sequencing. Unlike other kits in the markets, our patented plasmid purification kit has no chaotropic salts in the buffer. The purified DNA is guanidine/anion exchange resin residues free.

II. Sample Type: For fast and efficient purification of plasmid DNA from 100-250 mL of *E. coli* culture.

III. Kit Contents:

Components	K1319-10 10 preparations	K1319-25 25 preparations	Part Number
ezBind Columns	10	25	K1319-XX-1
Filter Syringe	10	25	K1319-XX-2
Buffer A1	120 mL	260 mL	K1319-XX-3
Buffer B1	120 mL	260 mL	K1319-XX-4
Buffer C1	140 mL	320 mL	K1319-XX-5
RNase A	420 µL	900 µL	K1319-XX-6
Elution Buffer	15 mL	50 mL	K1319-XX-7
DNA Wash Buffer*	50 mL	2 X 50 mL	K1319-XX-8
Endo Clean Buffer	10 mL	15 mL	K1319-XX-9

Add 200 ml of 100% ethanol to each DNA Wash Buffer*

IV. User Supplied Reagents and Equipment:

- 70% ethanol and 100% ethanol.
- High speed centrifuge.
- 30 mL high speed centrifuge tubes.
- 50 mL conical tubes.

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). RNase A should be stored at 4ºC. 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 is stable for more than half a year when stored at RT. 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.
- 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 room temperature.

VII. <u>Plasmid Maxiprep Spin Protocol:</u> 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 **150-200 mL LB** containing appropriate antibiotic with 100 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 6-8 hr with vigorous shaking (~250 rpm). Note: Do not use more than 250 mL culture or cell mass greater than 550. The buffer volume needs to be scaled up if processing over 250 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 room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium completely. *Note: Complete removal of residue medium is critical for bacteria lysis in the next step.*
3. Add **10 mL Buffer A1** ***(Add RNase A to Buffer A1 before use)*** and completely resuspend bacterial pellet by vortexing or pipetting. *Note: Complete resuspension is critical for optimal yields.*
4. Add **10 mL Buffer B1**, mix gently but thoroughly by inverting 5-10 times. If necessary, continue inverting the tube until the solution becomes slightly clear. Incubate at RT 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 **4.0 mL Buffer C1**, mix immediately by inverting 1o times and sharp shaking the vial for 3 times.
6. Two options for clearing the lysates: **Option 1:** Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm for 10 min at RT. Carefully transfer the clear supernatant into a 50 mL tube (avoid the floating precipitates). Add **8 mL Buffer C1** and mix well and go to step 8. **Option 2:** Allow the cell lysate to sit for 8 min. Add **8 mL Buffer C1** and mix well by inverting 2 times. Set for 1 min. The white precipitates should float to the top. Set the filter syringe barrel at a column holder or tape the syringe barrel to a clean 50 mL tube. Transfer the relatively clear lysate from the bottom of the tube to the barrel of the syringe, avoid the major preparations. Gently insert the plunger to expel the cleared lysate to the clean 50 mL tube.
7. Attach the maxi column to a vacuum manifold according to manufacturer's instruction.
8. **Add 10 mL absolute ethanol (96-100**% ethanol) to the clear lysate. Mix immediately by sharp shaking for 2 min. The mixture of ethanol/lysate needs to be centrifuged through the DNA column immediately.

9. Immediately transfer **20 mL of the lysate/ethanol mixture** to the column, apply vacuum to allow sample to pass through the column. Add the remaining lysate/ethanol mixture to the DNA column till all the sample has been passed through the column.
10. Add **10 mL 70% ethanol** into the column all allow the liquid to pass through the column by vacuum Repeat once.
11. Turn on the vacuum to dry the column for 15-20 minutes. *This step removes ethanol residues.* **Note***: Increase the time to 25 minutes if over 10 samples are being processed*. **Note:** Alternatively, the column can be dried by centrifugation in a swing-bucket rotor for 10 minutes.
12. Turn off the vacuum and detach the column from the manifold, tap the column tip on layers of clean paper towel. Wipe off any trace ethanol inside the column. Transfer the column to a clean 50 mL conical tube.
13. Add *1.0-2.0 mL Elution Buffer* to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 5,000 x g for 1 min. Add the eluted DNA back to the column, incubate for 1 min, and then elute the DNA by centrifugation at 5,000 x g for 3 min. The first elution normally yields 60-70% of the DNA bound. Add the eluted DNA back to the column and centrifuge at 5,000 x g for 3 min. The second elution yields another 20% of the DNA bound.
14. Two elution give rise to maximum DNA yield. Use less elution buffer if higher DNA concentration is desired. For maximum DNA yield, elute twice with 2 mL of Elution Buffer, precipitate the DNA, and resuspend DNA in Elution Buffer. ***Note:*** *The DNA is ready for downstream applications such as cloning or transfection of HEK293 cells.* ***Note****: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.*

VIII. Endotoxin Removal Procedure:

This protocol is designed to remove endotoxin after the plasmid DNA is purified (Buffers can be scaled up or down accordingly).

1. Add 0.1 volume of EndoClean buffer to the plasmid sample in a 1.5 mL sterile tube. (For example, add 0.1 mL endoClean buffer to 1 mL plasmid sample.
2. Mix by vortexing the tube a few times and put on ice for about 10 minutes until the solution is clear without turbidity. (Rocking the sample in a cold room for 10 minutes is recommended if it is available).
3. Mix well again by inverting the tube a few times.
4. Incubate the tube at 37-55 °C water baths for about 2 minutes and the solution shall be turbid.
5. Centrifuge at top speed at room temperature for 3 minutes.
6. Carefully transfer the upper clear layer solution to 2.0 mL tube.
7. Precipitate plasmid DNA with 0.1 volume of 3 M KAc (pH 5.2) and 0.7 volume of 100% isopropanol. Mix well.
8. Spin at 13,000 rpm for 10 min. Decant and add 500 µL 70% ethanol. Centrifuge at 13,000 rpm for 5 min. Decant.
9. Dry the DNA in a speedvac for 5-10 min or airdry the sample at a TC hood till DNA is completely dry. Resuspend the DNA in Endofree water.

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: 0.2 N NaOH and 1%SDS). • Grow bacterial 12-16 hrs. 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, C1 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. Re-centrifuge or vacuum again if necessary.

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