Plasmid ezFilter Mega10 Kit

(Catalog # K1322-1, -2, -10; Store at multiple temperatures)

I. Introduction:

BioVision's Plasmid ezFilter Mega10 Kit is designed for fast and efficient purification of 10-12 mg plasmid DNA within an hr from 1500 ml of *E. coli* culture. Unlike other procedures, our patented plasmid purification kit has no guanidine salt in the buffer, the purified DNA is guanidine/ion exchange resin residues free which enable the high performance of downstream applications such as transfection, RFLP, DNA amplification, and automated sequencing.

II. Sample Type:

For fast and efficient purification of plasmid DNA from 1500 ml of *E. coli* culture.

III. Kit Contents:

Components	K1322-1 1 preparation	K1322-2 2 preparations	K1322-10 10 preparations	Part Number
DNA Unit	1	2	10	K1322-XX-1
Filter Unit	1	2	10	K1322-XX-2
Replacement Cup	1	4	20	K1322-XX-3
Buffer A1	110 mL	210 mL	2 X 530 mL	K1322-XX-4
Buffer B1	110 mL	210 mL	2 X 530 mL	K1322-XX-5
Buffer C1	130 mL	250 mL	3 X 450 mL	K1322-XX-6
RNase A (20 mg/mL)	11 mg (550 µL)	21 mg (1.1 mL)	120 mg (4 X 1.5 mL)	K1322-XX-7
Elution Buffer	30 mL	60 mL	270 mL	K1322-XX-8

IV. User Supplied Reagents and Equipment:

- 70% ethanol and 100% ethanol.
- Pump-driven vacuum system, 500 mL bottle or 1,000 mL bottle or equivalent pyrex glass bottles.
- 50 mL conical tubes.

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. 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 B1 precipitates below room temperature. 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.
- The proper volume of buffer ratio of A1:B1:C1: 100% ethanol = 1:1:1.2:1.2.
- 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. Plasmid ezFilter Mega10 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 1200-1500 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 2,000 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 1200-1500 mL overnight bacterial cells by centrifugation at 5000g 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 100 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).
4. Add 100 mL Buffer B1, mix gently but thoroughly by inverting 10 times and incubate at room temperature for 5 min to obtain a cleared lysate. *Note: 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.*
5. Add 120 mL Buffer C1 and mix immediately by inverting 5 times and sharp hand shaking for 10 times till a flocculent white precipitate forms. Incubate the mixture at room temperature for 10 min. *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 80 mL lysate can be filtered through the filter unit within 10-15 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 1000 mL standard bottle and screw tight. Connect the DNA unit to the vacuum with the vacuum off. Add 120 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. Repeat this step once.
12. Add 80 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 to remove the ethanol residues. *Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.*
13. Turn off the vacuum, wait for 1 min, and replace the 500 mL or 1000 mL bottle with a sterile 50 mL conical tube, screw tight.
14. Add 12 mL sterile ddH_2O or Elution Buffer evenly to the membrane and incubate for 2 min. Turn on vacuum to elute DNA. Typically, 5-6 mL of DNA containing 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 12 mL sterile ddH_2O or Elution Buffer and incubate for 1 min. Turn on the vacuum and collect the 2nd elution, typically 8-10 mL of solution can be collected. *Note: If ddH_2O is used for eluting DNA, make sure the pH is ≥ 7.0. Note: It is highly recommended to remove the endotoxin if the DNA is used for 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 KAc or NaAc (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 min in a tissue culture hood. Resuspend the DNA in Elution Buffer or sterile ddH_2O.*

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

VIII. Purification of Low-Copy-Number Plasmid and 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 2 X volumes of the high copy number culture.
2. Use 2X volume of the Buffer A1, Buffer B1 and Buffer C1 and 100% ethanol.
3. Use same volume of Wash Buffer (70% ethanol and 100% ethanol) and Elution Buffer.

IX. Related Products:

Product Name	Catalog Number
Plasmid Miniprep Kit I	K1312-50
Plasmid Miniprep Kit I	K1312-250
Plasmid Miniprep Kit II	K1313-50
Plasmid Miniprep Kit II	K1313-250
Plasmid Midi Kit I	K1314-2
Plasmid Midi Kit I	K1314-10
Plasmid Midi Kit I	K1314-25
Plasmid Midi Kit II	K1315-2
Plasmid Midi Kit II	K1315-10
Plasmid Midi Kit II	K1315-25
Plasmid ezFilter Midi Kit I, Centrifuge	K1316-2
Plasmid ezFilter Midi Kit I, Centrifuge	K1316-10
Plasmid ezFilter Midi Kit I, Centrifuge	K1316-25
Plasmid ezFilter Midi Kit II, Centrifuge	K1317-2
Plasmid ezFilter Midi Kit II, Centrifuge	K1317-10
Plasmid ezFilter Midi Kit II, Centrifuge	K1317-25
Plasmid ezFilter Maxi Kit	K1319-2
Plasmid ezFilter Maxi Kit	K1319-10
Plasmid ezFilter Maxi Kit	K1319-25
Plasmid ezFilter Mega3 Kit	K1320-1
Plasmid ezFilter Mega3 Kit	K1320-2
Plasmid ezFilter Mega3 Kit	K1320-10
Plasmid ezFilter Mega6 Kit	K1321-1
Plasmid ezFilter Mega6 Kit	K1321-2
Plasmid ezFilter Mega6 Kit	K1321-10

Plasmid ezFilter Mega10 Kit	K1322-1
Plasmid ezFilter Mega10 Kit	K1322-2
Plasmid ezFilter Mega10 Kit	K1322-10
Express Plasmid Midiprep Kit (25 min)	K1323-2
Express Plasmid Midiprep Kit (25 min)	K1323-10
Express Plasmid Midiprep Kit (25 min)	K1323-25
Express Plasmid Maxiprep Kit (25 min)	K1324-2
Express Plasmid Maxiprep Kit (25 min)	K1324-10
Express Plasmid Maxiprep Kit (25 min)	K1324-25
96-well Plasmid ezFilter Mini Kit	K1325-100 (1 Pack)
96-well Plasmid ezFilter Mini Kit	K1325-100 (4 Packs)

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 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.