96-well Plasmid ezFilter Mini Kit

(Cat# K1325-96-1; -4; Store at multiple temperatures)

I. Introduction:

BioVision's 96-well Plasmid ezFilter Mini Kit is designed for the parallel preparation of 96 plasmid minipreps within 1 hr. The purified DNA is ready for downstream applications such as DNA amplification, automated sequence, RFLP, transfection, and viral packaging.

- It is fast and reliable.
- 96 DNA preps can be done in less than 40 min.
- One-step filter-binding, which omits lysate transferring from plate to plate.
- 8-12 µg DNA per well for high copy plasmid from 1.5 mL culture.
- Can be adapted to many automated platforms.

II. Sample Type: For the parallel preparation of 96 plasmid minipreps within 1 hr.

III. Kit Contents:

	K1325-96-1 (1 Pack)	K1325-96-4 (4 Packs)	
Components	**96 preparations**	**96 X 4 preparations**	**Part Number**
96-Well DNA Plate	1	4	K1325-96-X-1
96-Well Lysate Clearance Plate	1	4	K1325-96-X-2
Ventilating Film	1	4	K1325-96-X-3
Sealing Film	4	16	K1325-96-X-4
Buffer A1	55 mL (Add RNase A before use)	110 mL	K1325-96-X-5
Buffer B1	55 mL	110 mL	K1325-96-X-6
Buffer N1	60 mL	160 mL	K1325-96-X-7
DNA Wash Buffer*	50 mL	3 X 50 mL	K1325-96-X-8
KB Buffer	55 mL	75 mL	K1325-96-X-9
RNase A	200 µL	400 µL	K1325-96-X-10
Elution Buffer	25 mL	70 mL	K1325-96-X-11

Add 200 mL (K1325-96-1 (1 pack)) and 800 mL (K1325-96-4 (4 packs)) of 96-100% ethanol to each bottle before use.

IV. User Supplied Reagents and Equipment:

- Centrifuge with swing-bucket rotor (4,000g).
- Vacuum pump capable of achieving 300-400 mbar.
- Standard vacuum manifold.
- Oven or incubator preset to 70°C.
- 96-well 2.0 mL deep well plates.

V. Shipment and Storage: All the reagents are shipped at room temperature. Except the Buffer A1/RNase A, which is stored at 4°C, all other components are stored at room temperature. The guaranteed shelf life is 24 months from the date of purchase. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

- Briefly spin down the RNase A vial and add the RNase A to Buffer A1.
- Dilute DNA Wash Buffer as follows:
- K1325-100 (1 Pack): Add 200 mL 96-100% ethanol to each bottle before use.
- K1325-100 (4 Packs): Add 800 mL 96-100% ethanol to each bottle before use.

VII. 96-well Plasmid ezFliter Miniprep 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 to 5 times.

Vacuum Manifold Protocol:

1. **Culture and harvest bacterial cells:** Inoculate 1.0-1.2 mL LB/antibiotics medium in a 96-well 2 mL plate with E.*coli* carrying desired plasmid and grow at 37°C for 18-20 hr.
2. Seal the plate with sealing film and pellet the bacterial by centrifugation at 1,500-2,000g for 5 min in a swing-bucket rotor at RT.
3. Remove the sealing film and discard supernatant. Tapping the inverted plate firmly in paper towel to remove excess medium. Resuspend the pellet in each well with 250 µL of Buffer A1/RNase A by vortexing or pipetting. No cell clumps should be visible after resuspension.
4. Wipe off any residual liquid on top of the plate. Add 250 µL Buffer B1 to each well and mix thoroughly by gentle shaking and rotating for 1 min. Incubate at RT for 2-3 min. The solution should become viscous and slightly clear. ***Note: Vigorous mixing will result in shearing the chromosomal DNA.***

5. Add 350 µL Buffer N1 to each well. Wipe off any buffer residues on the top of the plate and seal the plate with a sealing film. Mix by inverting the plate for 5 times and vortex for 5 sec. The flocculent white precipitate should form.

6. Assemble the vacuum manifold:

- Place the DNA plate into the plate holder inside the manifold
- Place the Lysate clearance plate on top of the manifold. (The DNA plate now should be positioned under the lysate clearance plate; some manifold may require internal height adjustment by 96-well collection plate).

7. Immediately transfer the lysate into the lysate clearance plate. Allow the lysate to stand for 5 min. The white precipitate should float to the top. Apply vacuum until all the lysate passes through the lysate clearance plate.

8. Turn off the vacuum and discard the lysate clearance plate. Carefully transfer the DNA plate that contains the cleared lysate to the top of the vacuum manifold and turn on the vacuum till all the lysate passes through the DNA plate.

9. Optional: Add 175 µL of Buffer KB to each well and apply vacuum till all the liquid passes through the DNA plate. Turn off the vacuum. *Note: Buffer KB is recommended for endA+ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from endA- strains such as Top 10 and DH5a.*

10. Add 750 µL of DNA Wash Buffer to each well and turn on vacuum till all buffer passes through the plate. Turn off vacuum. Repeat once.

11. Discard the waste in the manifold and dry the DNA plate with maximum vacuum power for 20 min.

12. Remove the DNA plate from the manifold and tap the plate on a stack of absorbent paper towels. Remove any residual moisture from the tip ends of the DNA plate with clean paper towel. **Optional:** Place the DNA plate into a vacuum oven preset at 70^{o}C for 10 min.

13. Place the DNA plate back to the vacuum manifold and apply maximum vacuum for another 5 min

14. Place the 96-well collection plate inside the manifold with the manifold adaptor and set the DNA plate on top of the manifold (Make height adjustment as necessary by adding another plate).

15. Add 100-150 µL Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile water to each well, let plate stand for 2 min. Apply maximum vacuum for 5-10 min to elute the DNA from the plate. Turn off vacuum and ventilate the manifold slowly. *Note: For maximum yield, add the eluted DNA back to the wells for a 2nd elution. The first elution normally yields 60-70% of the DNA while the 2nd elution yields another 20-30% of the DNA bound to the membrane.*

Centrifuge Protocol:

1. Culture and harvest bacterial cells: Inoculate 1.0-1.2 mL LB/antibiotics medium in a 96-well 2.0 mL plate with E.coli carrying desired plasmid and grow at 37^{o}C for 18-20 hr.

2. Seal the plate with sealing film and pellet the bacterial by centrifugation at 1,500-2,000g for 5 min in a swing-bucket rotor at RT.

3. Remove the sealing film and discard supernatant. Tapping the inverted block firmly in paper towel to remove excess medium. Resuspend the pellet in each well with 250 µL of Buffer A1/RNase A by vortexing or pipeting. Complete resuspension is critical for optimized plasmid yields.

4. Add 250 µL Buffer B1 to each well and mix thoroughly by gentle shaking and rotating for 1 minute. Incubate at room temperature for 2-3 min. The solution should become viscous and slightly clear. *Note: Vigorous mixing will result in shearing the chromosomal DNA.*

5. Add 350 µL Buffer N1 to each well. Wipe off any buffer residues on the top of the plate and seal the plate with a sealing film. Mix by inverting the plate for 5 times and vortex for 5 sec. The flocculent white precipitate should form.

6. Place a lysate clearance plate on top of a 2 mL plate. Transfer the lysate into the lysate clearance plate and allow the lysate to sit for 10 min. White precipitate should float to the top at this point.

7. Place the clearance/deep well plates in a swing-bucket rotor and centrifuge at 3,000g for 5 min. Discard the lysate clearance plate.

8. Place a DNA Plate on top of a 96-well collection plate and transfer the cleared lysate into the DNA plate.

9. Place a DNA Plate on top of a 1.6 mL plate. Centrifuge at 3,000g for 5 min.

10. Discard the flow-through liquid and reuse the deep well plate for next step.

11. Optional: Add 175 µL Buffer KB to each well and centrifuge at 3,000g for 5 minu. Discard the flow-through liquid and reuse the deep well plate for next step. *Note: Buffer KB is recommended for endA+ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from endA- strains such as Top 10 and DH5a.*

12. Add 750 µL DNA Wash Buffer to each well and centrifuge at 3,000g for 10 min. Repeat once.

13. Remove the DNA plate from the manifold and tap the plate on a stack of absorbent paper towels. Remove any residual moisture from the tip ends of the DNA plate with clean paper towel.

14. Optional: Place the DNA plate into a vacuum oven preset at 70^{o}C for 10 min.

15. Place the DNA plate on top of a 96-well collection plate. Add 75-100 µL of Elution Buffer or Sterile Water to each well of the DNA plate. Let the plate stand for 2 min.

16. Centrifuge the plate at 3,000g for 5 min to elute the DNA. Note: The DNA recovery rate and concentration depend on the elution volume. For maximum yields, elute with 150 µL Elution Buffer although the DNA concentration will be lower.

VIII. 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)

IX. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low Yield	• Poor Cell lysis. • Bacterial overgrowth • Culture not fresh	• Do not use more than 2 mL of overnight culture. • Resuspend the cell pellet completely. Buffer B1 if not tightly capped may lead to poor cell lysis. Prepare fresh Buffer B1 follows: 0.2 M NaOH, 1% SDS • Do not grow Bacterial culture for more than 16 hr. • Use fresh culture and do not storage the culture at 4^{o}C.
No DNA	• Forget to add ethanol to the DNA wash	• Prepare the DNA wash buffer as instructed.
Chromosomal DNA contamination	• Over mixing after adding Buffer B1	• Do not vortexing or vigorously mixing after buffer B1 is added.
DNA flow out of agarose gel when loading	• Trace ethanol contamination	• Recentrifuge or vacuum again the plate as instructed if necessary.
RNA visible on agarose gel	• Forget adding the RNase A to Buffer A1	• Add RNase A to Buffer A1.
Lysate clearance plate clogged	• Lysate was not mixed well after adding Buffer N1	• Mix the lysate well by inverting the plate for 5 times and then vortex for 5 sec.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com