

BIO BASIC INC.

EZ-10 96 WELL SPIN COLUMN PLASMID DNA MINIPREPS HANDBOOK

BS4152 and BS415

Version 16.0
ISO9001 Certified

20 Konrad Cresent, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

Table of Content

Introduction **p4**

Storage **p4**

Principle **p4**

Applications **p4**

Features **p4**

Quality Control **p5**

Procedures **p5**

a) Centrifugation based procedure **p10**

Troubleshooting Guide **p11**

Other Kits Available **p12**

EZ-10 96 WELL SPIN COLUMN PLASMID DNA MINIPREPS

Components	BS4152 (2 plates)	BS415 (5 plates)
RNase A (10mg/ml)	0.5ml	1.2ml
Solution I[(A)]	25ml	60ml
Solution II[(B)]	50ml	120ml
Solution III	90ml	210ml
Wash Solution[(C)]	2x35ml	4x48ml
Elution Buffer[(D)]	15ml	30ml
96 Well Filter Plate	2	5
EZ-10 96 Well Binding Plate	2	5
Deep Well Collection Plate	4	10
96 Well Storage Plate	2	5
Sealing Film	10	25
Protocol	1	1

(A) Before use, add RNase A to Solution I. Solution I should be stored at 4°C for frequent use, or at -20 ºC for long term storage.

(B) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37 ºC.

(C) Before use, add 140 ml of 96-100% of ethanol to 35 ml Wash Solution. If the volume of Wash Solution has changed due to leaking during transportation, it is necessary to re-measure its volume, and adjust the volume of required ethanol accordingly (volume of added ethanol: volume of Wash Solution=4:1).

(D) Elution Buffer is 2.5 mM Tris-HCl pH8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 20% lower.

Storage

With the exception of the RNase A, the kit may be stored at room temperature. The RNase A should be stored at 4ºC. The kit is stable for 12 months at room temperature. For longer storage, keep all contents in cold place.

Principle

The EZ-10 96 Well Spin Column Plasmid DNA Purification kit provides a simple, efficient and automated high throughput method for plasmid DNA purifications. Plasmid DNA is selectively adsorbed in silica gel-based EZ-10 columns in the 96 Well Binding Plate and other impurities such as proteins, salts and nucleotides are removed. Plasmid DNA can be eluted in a small volume of Tris buffer.

Note

This kit is used for preparation of up to 10 µg of pure plasmid DNA in each well.

Applications

Plasmid DNA purification

Features

- ✓ Fast: Entire procedure takes approximately 45 minutes
- ✓ High quality: Purified DNA can be used in any downstream applications such as sequencing,

transformation, restriction enzymatic digestion, and ligation
- ✓ High yield (>80%) and reproducible
- ✓ Convenient and environmentally friendly. No phenol/chloroform extraction or ethanol precipitation required

Quality Control

All components in the kit are tested in purification of 96 x 10 µg pUC18 plasmid DNA from overnight culture.

Procedures

1. Fill each well of a Deep Well Collection Plate with 1.3 ml of growth medium containing the appropriate selective antibiotic. Inoculate each well from a single bacterial colony. Incubate the cultures for overnight or 20-24 hours at 37°C with shaking at 300 rpm.

Note: The wells in the block may be protected against spill-over by covering the block with a plastic lid or adhesive tape. If non-porous tape is used, pierce 2–3 holes in the tape with a needle above each well for aeration. For optimal DNA yield, use 1.5 - 5 ml overnight culture to start.

2. Harvest the bacterial cells in the Deep Well Collection Plate by centrifugation for 10 minutes at 5,788 Xg in a centrifuge with a rotor for microtiter plates. The Deep Well Collection Plate should be covered with Sealing Film during centrifugation. Remove medium by inverting the Deep Well Collection Plate.

Note: To remove the media, peel off the Sealing Film and quickly invert the Deep Well Collection Plate over a waste container. Tap the inverted Deep Well Collection Plate firmly on a paper towel to remove any residues.

3. Resuspend each well of bacterial cells in 100 µl Solution I. Tape the Deep Well Collection Plate with a new Sealing Film. Vortex for approximately 3 minutes.

Note: Ensure that RNase A has been added to Solution I. The pelleted cells in the Deep Well Collection Plate must be resuspended completely, leaving no cell clumps.

4. Add 200 µl of Solution II to each well, seal the Deep Well Collection Plate with a new Sealing Film, mix gently but thoroughly by inverting 10 times and incubate for 2 minutes.

Note: Do not vortex at this step to avoid shearing of the bacterial genomic DNA. Do not incubate for more than 5 minutes. Additional incubation can result in increased levels of open circular plasmid DNA. At the end of the incubation, the lysate should appear viscous and free of bacterial cell clumps. Avoid extended exposure of Solution II to air since CO_2 can reduce its effectiveness.

5. Add 350µl Solution III to each well, seal the Deep Well Collection Plate with Sealing Film, and mix immediately by inverting 10 times and incubate for 3 minutes.

Note: Gently inverting the taped Deep Well Collection Plate 10 times to ensure uniform

precipitation. To seal, completely dry the block with a paper towel, take a new Sealing Film and seal tightly either manually or using a sealing roller.

STEP 6A (OPTIONAL)

6A. Remove the Sealing Film from the Deep Well Collection Plate and add 300 µl isopropanol to each well. Seal the plate firmly with a new Sealing Film. Mix by inverting the plate 1–2 times. Further inversions are not required and may cause leakage of the isopropanol. Ensure that the plate is tightly sealed to avoid cross-contamination of wells. If the plate is loosely sealed, the isopropanol can cause the sealing film to detach from the plate.

Centrifugation Based Procedures

Note: For centrifugation based method, there is a minimum height requirement of 75 mm for apparatus to hold the assembly of EZ-10 96 Well Spin Column Plate and Deep Well Collection Plate.

6. Spin down at 4,500 x g for 15 minutes following Step 5.
7. Remove the tape. Assemble 96 Well Filter Plate on top of a new Deep Well Collection Plate. Pipette the supernatant from step 6 (650µl) into the center part of each well of 96 Well Filter Plate. Seal the plate with a new tape.

8. Centrifuge at 5,700xg for 5 minutes.

9. Place an EZ-10 96 Well Binding Plate on top of a new Deep Well Collection Plate. Transfer the above supernatants (clear lysate) from step 8 into EZ-10 96 Well Binding Plate using an 8-channel pipette. Centrifuge at 5,700 x g for 5 minutes.

10. Discard the flow-through in the Deep Well Collection Plate. Add 500 µl of Wash Solution to each well of EZ-10 96 Well Binding Plate, and centrifuge at 5,700 x g for 5 minutes.

11. Repeat step 9. (Optional: Repeat wash step one more time if needed)

12. Discard the flow-through in the Deep Well Collection Plate. Spin at 5,700 x g for additional 5 minutes to remove residual Wash Solution.

13. To elute, place a 96 Well Storage Plate on top of a Deep Well Collection Plate, and then place the EZ-10 96 Well Binding Plate on the top of a 96 Well Storage Plate. Add 50 µl of Elution Buffer into the center part of the membrane of each well and incubate at 37-50 ºC for 2 minutes. Spin at 4,500 x g for 5 minutes.

Note: 96 Well Storage Plate is very fragile and needs to be placed on top of a Deep Well Collection Plate for support during centrifugation.

14. Plasmid DNA is ready for use or store at –20 ºC freezer.

Troubleshooting

1. RNA contamination

a) RNase A digestion might be insufficient. Check the culture volume against recommended volumes, and adjust if necessary. If RNaseA solution is kept for more than 6 months, add more RNase A.

b) Prolong the standing time after adding supernatant to the EZ-10 96 Well Binding Plate.

2. Low yield of plasmid DNA
   Alkaline lysis is inefficient: If cells have grown to a very high density, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions. Reduce culture volume or increase volumes of Solution I, II and III.

3. For optimal results in downstream DNA sequencing, an additional washing step is recommended.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Other Kits Available

F505011-0001 Vacuum Manifold for 96-well Plate

96-Well Plate Plasmid DNA Mini-Preps Kit
(Vacuum Based)
B814152-0002 (2 Plates), B814152-0005 (5 Plates)

EZ-10 Spin Column Plasmid DNA MiniPreps Kit
BS413 (50preps), BS414 (100preps)

EZ-10 Spin Column PCR Products Purification Kit
BS363 (50preps), BS364 (100preps)

EZ-10 Spin Column DNA Gel Extraction Kit
BS353 (50preps), BS354 (100preps)

Please visit www.biobasic.com