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BIO BASIC INC.

96-Well Plate Bacterial Genomic DNA Mini-Preps Kit

SK1292 and SK1295

Version 9.0
ISO9001 Certified

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96-Well Plate Bacterial Genomic DNA Mini-Preps Kit

Product information for SK1292/SK1295:

Kit Contents

Components	SK1292 2 plates	SK1295 5 plates
Digestion Buffer(1)	160 ml	2X200 ml
TE(pH=8.0)	60 ml	150 ml
Proteinase K(2)	8 mg	20 mg
Wash Solution(3)	50 ml	3X48 ml
Elution Buffer	12 ml	30 ml
EZ-10 96 Well Binding Plate	2	5
Deep Well Collection Plate	4	10
96 Well Storage Plate	2	5
Sealing Film	8	20
Protocol	1	1

Note:

1. If there is precipitation in digestion buffer, warm up at 50^{o}C before use.
2. Before use, add 800 µl of sterilized water to 8 mg Proteinase K, add 2.0 ml of sterilized distilled water

to every 20 mg Proteinase K. Store it at -20 °C for future usage.

3. Before use, add 200 ml of anhydrous ethanol to 50 ml Wash Solution for SK1292 or 192ml of ethanol to 48 ml Wash Solution for SK1295. 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).
4. Elution buffer is 2.0 mmol/l Tris-HCl, pH 8.0~8.5. TE buffer (pH 8.0) or distilled water (pH>7.0) can be used to elute, however, the yield is generally lower.

Storage

Transportation at ambient temperature. Upon receipt, store kit at 4°C.

Introduction

The kit provides a simple and convenient high throughput approach to isolate high quality Genomic DNA from both Gram negative and Gram positive bacteria. DNA of cell lysate is selectively adsorbed on EZ-10 Spin Column, impurities such as proteins, salts and nucleotides are washed away. No phenol/chloroform extraction or ethanol precipitation is required. Purified genomic DNA can be up to 50 kb in length and can be used for PCR, and other downstream applications.

Feature

√ High purity: Prepared DNA can be used for PCR directly and other downstream applications.
√ Efficient: whole procedure only takes 60 minutes.
√ No phenol/chloroform extraction, no ethanol precipitations are required.

Procedures

1. Transfer 1.6 ml overnight bacterial culture per well into a Deep Well Collection Plate. Seal the plate with a Sealing Film. Centrifuge 2~3 minutes at 5,700 x g. Discard the supernatant.
2. Add 200 µl of TE to each well of the Deep Well Collection Plate. Seal it by a new Sealing Film. Vortex vigorously to mix well.
3. Add 400 µl of Digestion Buffer to each well, seal the plate with a new Sealing Film. Vortex and to mix well. Add 3 µl of Proteinase K to each well, seal and mix well. Incubate at 55 °C for 15~30 minutes until the solution becomes transparent and clear.
4. Add 260 µl of anhydrous ethanol to each well. Seal it with a new Sealing Film. Mix well by inverting the plate several times.

Centrifugation Based Procedures

5. Remove the tape. Assemble EZ-10 96 Well Filter Plate on top of a new Deep Well Collection Plate. Transfer the liquid from Step 4 to the EZ-10 96 Well Plate. Seal the plate with a new tape. Keep it at room temperature for 2 minutes.
6. Centrifuge for 2 minutes at 5,700 x g.
7. 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.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the Deep Well Collection Plate. Spin at 5,700 x g for additional 2 minutes to remove residual Wash Solution.
10. 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 55 °C for 2 minutes. Spin at 4,500 x g for 2 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.

11. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20 °C freezer.

Vacuum Based Procedures:

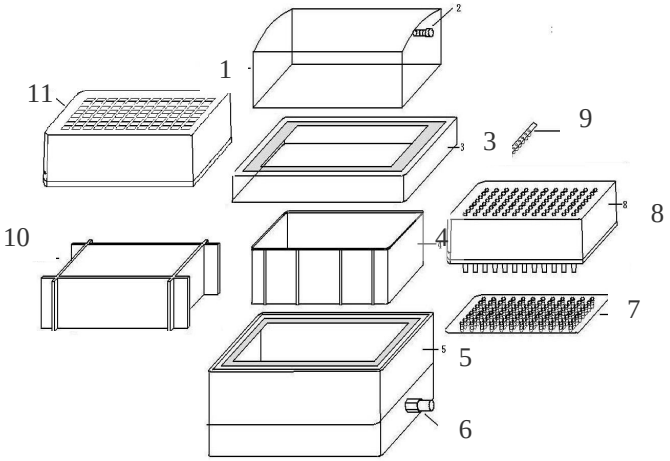

Figure 1. *Components of EZ-10 96 Well Spin Column Plasmid DNA Minipreps Kit*

1. Top Cap 2. Release Valve 3. Base Cap 4. Waste Tray 5. Base
6. Vacuum Connector 7. 96 Well Storage Plate 8. 96 Well Plate
9. 8 Well Strip Vacuum Sealer 10. 96 Well Storage Plate Holder 11. Deep Well Collection Plate

Note: Vacuum Manifold (SD5011 – including 1, 3, 4, 5, 10) and 8 Well Strip Vacuum Sealer (BP547) are sold separately.

5. Assemble the vacuum manifold. Place a Waste Tray in the Base; cover it with the Base Cap, and place a clean EZ-10 96 Well Plate on top.
6. Transfer the liquid from step 4 carefully into the wells of the EZ-10 96 Well Plate. Apply vacuum until all samples have passed through.
7. Switch off the vacuum and ventilate the vacuum manifold slowly. Add 500 µl of Wash Solution to each well and apply vacuum until buffer has passed through.
8. Repeat step 7.
9. After Wash Solution has been drawn through all wells, apply maximum vacuum for an additional minute to dry the membrane.
10. Switch off the vacuum and ventilate the vacuum manifold slowly. Remove the EZ-10 96 Well Plate together with the Base Cap from the Base. Vigorously tap the plate on a stack of absorbent paper, and blot the nozzles of the 96 Well Plate with clean adsorbent paper until no droplets remain.
11. For elution, assemble the vacuum manifold. Place the Storage Plate Holder in the Base, put 96 Well Storage Plate on top, and cover it with Base Cap. Place EZ-10 96 Well Plate on top securely. Mark the orientation appropriately.
12. To elute DNA, add 50 µl of Elution Buffer onto the center of each well of the 96 Well Plate, Incubate at 55 °C for 2~3 minutes, and apply vacuum (-550 to -650 mbar) for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.
13. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20 °C freezer.

Note: It is important to add the Elution Buffer into the center of each well.

Troubleshooting

1. Low yield

a. Insufficient lysis
   Increase the amount of Digestion Buffer and Protease K or prolong the incubation time.
b. Low temperature of Elution Buffer
   For step 10(Centrifugation method) or step 12 (vacuum procedure), keep the 96 Storage Plate at 55 ^{o}C for a longer period of time.

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

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