



# **PRODUCT INFORMATION**

## **96-WELL PLATE CLASSIC PLASMID DNA MINIPREPS KIT**

### **Kit Contents**

<b>Components</b>	<b>BS417 , 5 Plates</b>	<b>BS418 , 10 Plates</b>
RNase A(10mg/ml)	3X1.2ml	6X1.2ml
Buffer I	180ml	2X180ml
Buffer II	180ml	2X180ml
Buffer III	180ml	2X180ml
Sterile H <sub>2</sub> O	30ml	60ml
96 Filter Plate	5	10
Deep Well Collection Plate	10	20
Sealing Film	20	40
Protocol	1	1

- (a) Before use, add RNase A to Buffer I. Then Buffer I Should be stored at 4°C for frequent use ,or stored at -20°C for a long period use.
- (b) Buffer II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37°C.

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

Note: The purification method is based on centrifugation. There is a minimum height requirement of 750mm for apparatus to hold the assembly -- filter plate and deep well plate.

### **Principle:**

This 96-well filter plate kit provides a simple, fast, economic and automated high-throughput method to purify plasmid DNA from culture to DNA. The procedure is based on a modified alkaline lysis followed by effective filtration and precipitation. The procedure processes using both vacuum-driven transfer and centrifugation. Using 1.5ml rich medium up to 10ug plasmid DNA in each well can be obtained. This 96-well format kit is ideal for use in high-throughput automated sequencing projects. Compared with EZ-10 Spin column 96-well plate Mini-preps Kit, this 96-well Classic Plasmid DNA Mini-preps Kit is more economic.

### **Applications:**

- Fast, high-throughput procedure
- Multiwell format from culture to DNA

### **Procedure:**

Cultures grown in 96-well filter plate are harvested and lysed by a modified alkaline lysis system followed by filtration. Plasmid DNA in the filtrates is concentrated by isopropanol precipitation. Using 1.5ml of rich medium up to 10 ug of pure plasmid DNA in each well can be obtained. The purified plasmid DNA can be



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used for any downstream experiments such as DNA sequencing, restriction enzyme digestion, transformation and ligation

## **Procedure for Purification of Plasmid DNA**

1. Fill each well of a Deep Well Collection Plate with 1.3 ml of growth medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. Incubate the cultures for 20-24h at 37°C with shaking at 300 rpm.
2. Harvest the bacterial cells in the Deep Well Collection Plate by centrifugation for 5 min at 1500rpm in a centrifuge with a rotor for microtiter plates. The Deep Well Collection Plate should be covered with adhesive tape during centrifugation. Remove media by inverting the Deep Well Collection Plate.

**Note:** To remove the media, peel off the tape and quickly invert the Deep Well Collection Plate over a waste container. Tap the inverted Deep Well Collection Plate firmly on a towel to remove any remaining droplets of medium.

3. Resuspend each bacterial cells in the Deep Well Collection Plate in 250 ul Buffer I. Tape the Deep Well Collection Plate and mix by vortexing, and keep for 3 minutes.

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

4. Add 250 ul of Buffer II to each well, seal the Deep Well Collection Plate with new tape, mix gently but thoroughly by inverting 10 times. and keep for 2 minutes.

**Note:** Do not vortex at this step, as this may cause 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. 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<sub>2</sub> can reduce the effectiveness of this solution.

5. Add 250 ul Buffer III to each well, seal the Deep Well Collection Plate with new tape, and mix immediately by inverting 10 times and keep for 3 minutes.

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

6. Spin down at 8,000 rpm for 15 minutes.
7. Place a 96-filter plate on the top of a Deep Well Collection.
8. Transfer the above supernatants (step 6) to 96-filter plate using an 8-channl pipet. centrifuge at 3,000 rpm for 5 minutes.
9. Add 700 ul of room temperature isopropanol Seal the Deep well collection plate , then vetox gently 5 times. and centrifuge at 8,000 rpm for 15 minutes.
10. Discard the flow-through in the Deep well collection plate.
11. Add 0.5ml 70% ethanol to the Deep well collection plate , Spin at 8,000 rpm for 5 minutes.
12. Repeat step 11.
13. Place the Deep well collection plate at 60°C to dry the remains ethanol.
14. Add 50 ul of Sterilized H<sub>2</sub>O into the low part of the Deep well collection plate of each well and incubate at 37- 50°C for 2 minutes. Then votex gently ,and Spin at 3,000 rpm for 5 minutes.



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15. Plasmids are ready for use or store at  $-20^{\circ}\text{C}$  freezer.