Endotoxin-Free **Plasmid Maxiprep Kit**

Cat. #: W2104-10 (10 reactions)

Storage: room temperature. After adding RNase A, store Buffer P1 at 2 ~ 8°C, and it is stable for 6 months. Other buffers and RNase A stock solution can be stored at room temperature for 12 months.

Product Description (This product is for research use only.)

Eukaryotic cells are very sensitive to endotoxin, a common contaminant in the plasmid extraction, and the transfection efficiency of the cells will greatly reduce if the plasmid contains endotoxin. This kit can efficiently extract endotoxin-free plasmid in a fast and simple way. The endotoxin, genomic DNA, RNA, protein and other impurities can be largely removed.

200 µg ~ 1.5 mg of **transfection-grade plasmid DNA** can be purified from 150 ~ 300 mL overnight culture of E. Coli in LB medium with this kit within 50 minutes.

The extracted DNA can be directly used for transfection, PCR, restriction enzyme digestion, sequencing, etc. No need to precipitate, concentrate or desalt.

Product Components

Components	Amount	Storage
Buffer P1	125 mL	room temperature, store at 4°C if RNase A is added.
Buffer P2	125 mL	room temperature
Buffer E3	125 mL	room temperature
Buffer PS	30 mL	room temperature
Buffer PW (concentrate)	50 mL	room temperature
Endotoxin-Free Buffer EB	30 mL	room temperature
RNase A (10 mg/mL)	2 mL	room temperature
Plunger	10	room temperature
Endotoxin-Remover Filter	10	room temperature
Spin Column DQ (with collection tube)	10	room temperature
Collection Tube	10	room temperature

Note:

1. All reagents can be stored at room temperature (15 ~ 25°C) for 12 months. The Spin Column DQ (with collection tube) can be stored at 4°C for longer time. After adding RNase A, store Buffer P1 at 2 ~ 8°C, and it is stable for 6 months.
2. Add all the provided RNase A to Buffer P1 before use, shake well and store at 2-8°C. Before use this mixed Buffer P1, put it at room temperature for a while to warm it up to room temperature for better effect.

3. Before use Buffer PW for the first time, add ethanol (100%) to Buffer PW (according to the instructions on bottle label).
4. Check Buffer P2 and Buffer E3 before each use to see if any crystal or precipitate. Dissolve any crystal or precipitate by warming the Buffers in 37°C water bath, then the buffers will become clear again.
5. Buffer P2 and Buffer E3 are irritating. Handle with gloves. Fasten bottles tightly immediately after each use.
6. After equilibrated by Buffer PS, use the Spin Column DQ as soon as possible. Sitting too long will decrease the effectiveness.
7. The plasmid DNA yield and purity are correlated to bacteria concentration, bacteria strain, plasmid DNA size and copy number of plasmid. The volume of bacterial culture needed depends on the concentration of cultured cells.
8. All protocol steps should be carried out at room temperature (15 ~ 25°C)

Protocol:

1. Harvest 150 mL overnight bacterial culture in a centrifuge tube (not provided), and centrifuge at 12,000x g for 3 minutes to pellet bacterial cells. Aspirate the supernatant completely.

   The volume of bacterial culture needed depends on the concentration of cultured cells. For long plasmid (>10 kb) and low-copy plasmid, centrifuge twice to harvest more bacterial cells (do not exceed 300 mL culture). Double the amount of buffer P1, P2 and E3 in the following reaction.

   The amount of the reagents must be able to fully lysis the bacteria cells, otherwise the extraction efficiency will be reduced.

2. Aspirate the supernatant completely. Invert the tubes on a paper towel to remove/drain all trances of medium.

3. Add **12 mL Buffer P1** (**check if RNase A has been added**) to the pelleted bacterial cells and resuspend thoroughly by vortex or pipetting up and down till no cell clumps remain.

   Note: If bacteria are not thoroughly mixed, the lysis will not be complete, and the plasmid yield and purity will be low.

4. Add **12 mL Buffer P2** and mix thoroughly by gently inverting the tube for 8 ~ 10 times to lysis the bacteria cells completely. **Stand for 3 ~ 5 minutes** at room temperature. The solution should become clear and viscous.

   Note:
   - Close Buffer P2 bottle immediately after use.

- **Do not allow the lysis reaction to proceed for more than 5 minutes.**
- **Do not shake vigorously or vortex, to avoid shearing of genomic DNA. If the solution is not clear, it suggests that the bacteria cells are excessive. Reduce the input amount of bacteria.**

5. Add **12 mL Buffer E3** and mix immediately and thoroughly by inverting the tube 8 ~ 10 times. White flocculent precipitate forms and the lysate becomes less viscous.

   Note: Mix thoroughly and immediately after addition of Buffer E3 to avoid localized precipitation.

6. Sit at **room temperature for 5 minutes**. Centrifuge at **12,000x g for 10 minutes**.

7. **Pour all supernatant to Endotoxin-Remover Filter**, slowly push the Plunger handle into filter, and collect the flow-through in a clean 50 mL centrifuge tube (not provided).

8. Add **11 mL isopropanol (0.3 volume) to the filtered lysate** and mix by inverting the tube 4 ~ 6 times.

   Note: excessive volume of isopropanol will cause RNA contamination.

9. **Column Equilibrate:** add **2 mL Buffer PS** to the Spin Column DQ in collection tube, centrifuge at 12,000x g for 2 minutes. Discard the flow-through and put the column back to the collection tube.

10. Transfer the mixture of the lysate and isopropanol from step 8 to the equilibrated column. Centrifuge at 6,000 ~ 13,000x g for 2 minutes. Discard the flow-through and put the Column back to collection tube.

    Note: **The maximum volume of the Spin Column DQ is 15 mL. Repeat this step for 3 ~ 4 times to run all the solution through the Column DQ.**

11. Add 10 mL Buffer PW (check if ethanol is added) to the spin column DQ, and centrifuge at 6,000 ~ 12,000x g for 2 minutes. Discard the flow-through.

12. Repeat step 11 once (wash twice).

13. Put the Spin Column DQ back into the collection tube and centrifuged at **12,000x g for 5 minutes** to remove residual wash buffer. Discard the flow-through and air dry the Column at room temperature for a few minutes to dry thoroughly.

    Note: The residual ethanol will affect the subsequent enzymatic reaction (digestion, PCR, etc.). Residual PW buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation.

14. Place the Spin Column DQ in a new collection tube. **Add 1 ~ 3 mL Endotoxin-Free Buffer EB** to the center of the Column. Let **stand for 2 ~ 5 minutes** at room temperature and **centrifuge at 12,000x g for 5 minutes** to collect the flow-through. **The flow-through is purified plasmid DNA**. Use it directly or stored at -20 ℃.

Note:

1) In order to increase the recovery of plasmid, the obtained solution can be re-added to the spin column DQ and repeat step 14. **For low copy plasmid or plasmid size > 10kb, preheat Endotoxin-Free Buffer EB in 65 ~ 70 ℃ water bath** and extend the adsorption and elution time will increase the yield.

2) The pH value impacts the elution efficiency significantly. If use ddH_2O to elute the DNA, the pH value should be kept between 7.0 and 8.5 (adjust the pH of the ddH_2O with NaOH as needed). If the pH value below 7.0 the elution efficiency reduces greatly. The elution volume should not be less than 1 mL.

-- The end --

Related products

Cat.#	Kit Name	Application		Protein Status	min.
P501	Total protein kit	cells	Total protein	Denatured / Native	1 ~ 8
P502	Total protein kit	tissues	Total protein	Denatured / Native	1 ~ 8
P503	Membrane protein kit	cells / tissues	PlasmaMembrane	Native & Detergent-free	20 ~ 45
P504	Nuclear protein kit	cells / tissues	Nuclear & cytosol protein	Native	6 ~ 8
P505	Detergent-free kit	cells	Total protein	Denatured / Native	5 ~ 8
P506	Detergent-free kit	Tissues	Total protein	Denatured / Native	5 ~ 8
P507	Mitochondria kit	cells / tissues	Mitochondria	Native & Detergent-free	25 ~ 30
P508	Plant total protein	plant tissues	Total protein	Denatured/Native	5 ~ 8
P510	Plant detergent-free	plant tissues	Total protein	Native	6 ~ 8
P511	Plant chloroplast kit	plant tissues	Intact chloroplast		5
P512	Bacteria total protein	bacteria	Total protein	Denatured	2 ~ 3
P513	Nuclear envelope kit	Cells	Nuclear envelope	Native	< 45
P514	Histone/DNA binding protein extraction kit	Cells	Histone & DNA binding protein	Denatured	< 10
P515	Thick cell wall microbes kit	Microbes	Total protein	Denatured / Native	< 10
P519	Gel slice recovery kit	PAGE gel	Protein	Denatured / Native	10 ~ 20
P521	Hair & nail protein kit	Hair, nail	Protein	Denatured	5 min. hands on
P522	Adipose protein kit	Adipose/adipocyte	Total Protein	Denatured / Native	20