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DATA SHEET

Version: 03
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1. Identification

Product name **WideUSE™ Plasmid Maxiprep Kit**
10 reactions

Cat. No **AN0169**

2. Description

WideUSE plasmid Maxiprep kit offers a simple and convenient method for the routinary isolation of high-quality plasmid preparations in Maxiprep format.

3. Components

Item	Quantity
Plasmid Maxi Column	10
Equilibration Buffer	135mL
Resuspension Solution	215mL
Lysis Solution	215mL
Neutralization Solution	215mL
Wash Buffer	2 x 165mL
Elution Buffer (EB)	215mL
RNase A (lyophilized)	21,5mg

4. Storage

Store RNase A at -20 ˚C upon recipe kit.
If precipitates have formed in Lysis solution, warm the buffer in 37˚C water bath to dissolve precipitates.
Store rest of components of the kit at room temperature.

5. Specifications

- ✓ **Technology:** Anion-exchange chromatography (gravity-flow column)
- ✓ **Lysate clarification:** centrifugation
- ✓ **Sample Size:** 120 ~ 240 ml of bacteria for high-copy number or low-copy number plasmid
- ✓ **Plasmid or constructs range:** 3kbp ~ 150kbp
- ✓ **Binding Capacity:** 1,5 mg / Maxi Column

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6. Further information

Product Use Limitations	This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Safety Information	When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.

PROTOCOL

Before start:

1. **Adding the provided RNase A to Resuspension Solution:**
   add 0.5 ml of Resuspension Solution to a RNase A tube, vortex the tube to dissolve the RNase A completely. Transfer the total RNase A mixture back to the Resuspension Solution bottle, mix well by vortexing and store the Resuspension Solution buffer at 4˚C.
   After RNase A has been added, mark the bottle to indicate that this step has been completed.
2. If precipitates have formed in **Lysis Solution**, warm the buffer in 37˚C water bath to dissolve precipitates.
1. Pre-chill **Neutralization Solution** at 4 ˚C before starting.

Harvest bacterial cells:

1. Harvest the cells by centrifugation at **4,500 ~ 6,000 x g at 4 ºC for 10 min** and discard the supernatant.

Equilibrate Plasmid Maxi Column:

2. Place a Maxi Column onto a 50 ml tube.
3. Equilibrate the Plasmid Maxi Column by applying **10 ml of Equilibration Buffer**. Allow the column to empty by gravity flow and discard the filtrate.

Cell lysis and lysate neutralization:

4. Add **16 ml of Resuspension Solution** (RNase A added) to resuspend the cell pellet by vortexing or pipetting. *Make sure cell pellet be suspended completely.*
5. Add **16 ml of Lysis Solution** and mix gently by inverting the tube 5 times.
   Do not vortex to avoid shearing genomic DNA.
6. Incubate the sample mixture for 5 minutes at room temperature until lysate clears.
7. Add **16 ml of chilled Neutralization Solution** and mix immediately by inverting the tube 10 ~15 times to neutralize the lysate. **(Do not vortex!)**
   Notes:
   - Make sure the density of cultured cell is optimal, the buffers volume (Resuspension Solution, Lysis Solution, Neutralization Solution) should be increased proportionally to the culture volume.
     (ex. culture volume, 60 ~ 120 ml: 8 ml of each reagent.
     culture volume, 120~ 240 ml: 16 ml of each reagent.)
   - Mix the sample mixture completely after adding Lysis Solution and Neutralization Solution.

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Clarify lysate by centrifugation:

8. Centrifuge the tube at ≥ **5,000 x g at 4 ˚C for 20 min**. preferably centrifuge the tube at **15,000 ~ 20,000 x g at 4 ˚C for 15 minutes.**
   If the supernatant still contains suspended matter, transfer the supernatant to a clean centrifuge tube and repeat this centrifugation step.
9. Transfer the supernatant to a clean 50 ml tube.

Binding of plasmid:

10. Transfer the supernatant from step 9 to the **equilibrated Plasmid Maxi Column**. Allow it to flow through the Plasmid Maxi Column by gravity flow and discard the filtrate.
11. Repeat step 10 for the rest of the supernatant.

Wash Plasmid MaxiColumn:

12. Wash the Plasmid Maxi Column by applying **30 ml of Wash Buffer**. Allow Wash Buffer to flow through the Plasmid Maxi Column by gravity flow and discard the filtrate.

Elution:

13. Place the Plasmid Maxi Column onto a clean 50 ml centrifuge tube (not provided). Add **15 ml of Elution Buffer** to the Plasmid Maxi Column to elute the plasmid by gravity flow.

Precipitate plasmid DNA:

14. Transfer the elute from step 13 to a centrifuge tube. Add **0.75 volumes** of room temperature **isopropanol** (Not included) to the eluate and mix well by inverting the tube 10 times. (ex: add 11.25 ml isopropanol to 15 ml eluate)
    Note! Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.
15. Centrifuge the tube at **≥ 5,000 x g at 4 ˚C for 30 min**. preferably centrifuge the tube at **15,000 ~ 20,000 x g at 4 ˚C for 20 minutes.**

Wash and dissolve plasmid DNA

16. Carefully remove the supernatant and wash the plasmid pellet with **5 ml** of room temperature **70% ethanol.**
17. Centrifuge the tube at **≥ 5,000 x g at 4 ˚C for 10 min.**
18. Carefully remove the supernatant and invert the tube on paper towel for 3 minutes to remove residual ethanol. Air-dry the plasmid pellet until the tube is completely dry. (Or incubate the plasmid pellet at 70 ˚C for 10 min.)
19. Dissolve the plasmid pellet in a **suitable volume of ddH2O.**
    Note:
    - Do not lose the DNA pellet when discard the supernatant.
    - Make sure the DNA pellet adhesive lightly on the centrifuge tube.
    - If the DNA pellet loose from tube, repeat the precipitation step again.
    - Make sure the DNA is dissolved completely before measure the concentration.