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DATA SHEET

Version: 03
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1. Identification

Product name **WideUSE™ Plasmid Purification MiniSpin Kit**

Cat. No **AN0068 (50 reactions)**
Cat. No **AN0069 (100 reactions)**
Cat. No **AN0069-XL (250 reactions)**

2. Description

The WideUSE™ Plasmid Purification Kit is designed for rapid and cost-effective small-scale preparation of high-quality plasmid DNA from recombinant E. Coli cultures. By combining silica-binding technology and the convenience of a spin column format, up to 24 µg of high copy plasmid DNA can be recovered from 1-5 mL of E. coli culture in less than 30 minutes. The extraction process includes an initial SDS/alkaline lysis step with the appropriate Buffer to liberate plasmid DNA from the cell followed by adsorption of the DNA onto silica in the presence of high salts. Contaminants are then removed by a spin-wash step. Finally, the bound DNA is eluted in water or Tris-EDTA buffer.

3. Kit Components

Item	Quantity		
	AN0068 (50 rxn)	AN0069 (100 rxn)	AN0069-XL (250 rxn)
WideUSE minispin columns	50	100	250
Collection tubes (2 mL)	50	100	250
S-I Buffer	8 ml	16 ml	40 ml
S-II Buffer	8 ml	16 ml	40 ml
S-III Buffer	8 ml	16 ml	40 ml
Binding Buffer	30 ml	60 ml	150 ml
Washing Buffer*	8 ml	16 ml	40 ml
Elution buffer	6 ml	10 ml	20 ml
RNase A	80 µl	160 µl	400 µl

*Add the volume ethanol (96%-100%) specified [Not included] to WB Buffer prior to initial use (see the label on the bottle for a volume indication). After ethanol has been added, mark the bottle to indicate that this step has been completed.

4. Features

- **High yields** of up **to 24 µg of DNA** suitable for all molecular biology procedures.
- **Efficient purification** of plasmids ranging from **1 to 15 kb**
- No phenol-chloroform extraction.
- **Ready to use** plasmid DNA.
- **Just a few minutes** procedure.
- **Mini format**

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5. Storage specifications

WideUSE™ Plasmid Purification Kit should be stored at room temperature (15–25˚C) for up to 24 months without any reduction in performance. Store the RNase A at -20°C. After addition of RNase A to Buffer S-I reagent can be stored at 4°C.

6. Applications

All molecular biology applications, such as:
- Digestion with restriction enzymes.
- Automated sequencing.
- PCR template.
- Bacterial transformation.

7. Quality Certifications

Plasmid Purification Kit is tested for the isolation of any plasmid DNA from transformed E.coli. The quality of purified DNA is analysed by:
- Ratio 260/ 280.
- Agarose gel electrophoresis.
- Digestion with restriction endonucleases.

8. PRODUCT USE LIMITATION

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.

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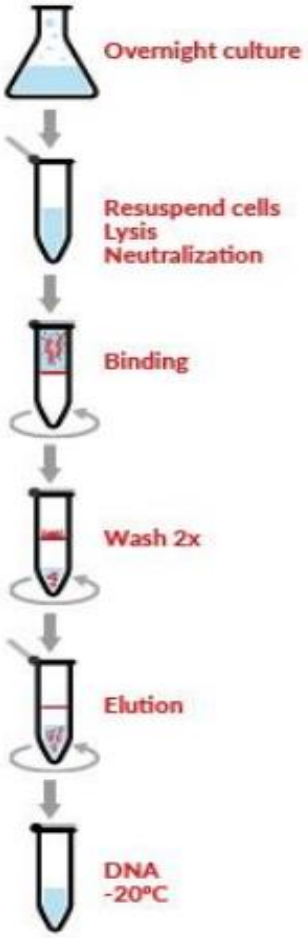
PROTOCOL

PREPARATION OF WORKING SOLUTIONS

Before starting the protocol prepare the following reagent:
✓ To prepare the S-I Buffer add the vial of **RNAse A.**
✓ Add the volume ethanol (96%-100%) specified [Not included] to Washing Buffer prior to initial use. After ethanol has been added mark the bottle to indicate that this step has been completed.

ASSAY PROCEDURE



1. Pour the culture in a 1.5 ml centrifuge tube and harvest the bacterial cells by centrifugation att 13,000 g for 2 minutes (For low-copy plasmids should be collect 3 mL of culture and using 2 volumes of each solution to obtain good yields).

   Repeat step 1 using the same tube at least once. Discard the supernatant completely
2. Resuspend the bacterial pellet in 100 µL of **Buffer S-I**
3. Add 100 µL of **Buffer S-II**, mix thoroughly by inverting the tube 6 times.
4. Incubate the mixture at room temperature about 2 minutes to lyse the cells. Do not exceed 5 minutes of lysis
5. Add 100 µL of **Buffer S-III**, mix thoroughly by inverting the tube 8 times.
6. Centrifuge at 13,000 g in a microcentrifuge for 5 min. Recover supernatant containing plasmid DNA promptly into a 1.5 ml centrifuge tube.
7. Add 500 µL of **Binding Buffer**, mix by inverting the tube several times. Incubate at room temperature for 5 min.
8. Apply the mix to the **WideUSE spin column** placed into a collection tube, by decanting or pipetting.
9. Centrifuge at 10,000 g for 30 seconds. Discard the flow-through.
10. Repeat steps 7-8 with the remaining mixture, if any.
11. Wash the **WideUSE spin column** by adding 700 µL **washing Buffer** and centrifuging at 10,000 g, 30 seconds. Discard the flow-through.
12. Centrifuge at 10,000 g, 1 minute. Discard the flow-through.
13. Place the **WideUSE spin column** into a new, labelled 1.5 microcentrifuge tube and pipet 50-70 µl **Elution buffer** directly into the membrane or pre-warm water. Close the cap and incubate for 1 minute at room temperature for 2 minutes.

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Prewarm Elution Buffer or Water at 70°C.

14. Centrifuge at 10,000 g for 1 minute to elute DNA.
15. Store a -20°C