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DATA SHEET

Version: 03
Revision date: 11/04/2023

1. Identification

Product name **HigherPurity™ Bacterial Genomic DNA Isolation Kit**

Cat. No **AN0066 (50 reactions)**
Cat. No **AN0067 (100 reactions**
Cat. No **AN0067-XL (250 reactions)**

2. Description

HigherPurity™ Bacterial Genomic DNA Isolation Kit provides a simple and convenient technique to isolate high quality DNA from both Gram negative and Gram positive bacteria. Extraction is based on spin filter columns.
It has been optimized specifically for isolating bacterial DNA from cell pellets after culturing. The extraction process includes an initial cell-wall lysis step with the appropriate enzyme to ensure efficient cell lysis and DNA release from the cell. Genomic DNA can be isolated from crude lysate by its ability to bind silica in the presence of high concentrations of chaotropic salts as guanidinium thiocyanate. The DNA is then washed and desorbed from the surface of the filter.

3. Kit Components

Item	Quantity		
	AN0066 (50 rxn)	AN0067 (100 rxn)	AN0067-XL (250 rxn)
Minispin columns	50	100	250
Collection tubes (2 mL)	100	200	500
BR-1 Buffer	15 ml	30 ml	75 ml
BLU Buffer	20 ml	40 ml	100 ml
WB1 Buffer	30 ml	60 ml	150 ml
WB2 Buffer*	6 ml	12 ml	30 ml
EB buffer	10 ml	20 ml	50 ml
Proteinase K**	30 mg	2 x 30 mg	5 x 30 mg
Lysozyme***	25 mg	2 x 25 mg	5 x 25 mg
RNase A Solution (10 mg/ml)	1 ml	2 x 1 ml	5 x 1 ml

*Add the volume ethanol (96%-100%) specified [Not included] to WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

**Dissolve Proteinase K in water (1.5 ml) to obtain a 20 mg/mL stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at – 20 °C until needed.

***Dissolve lysozyme in water (0.5 ml) to obtain a 50 mg/mL stock solution. The lysozyme solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed.

4. Quality Certifications

Bacteria Genomic DNA Isolation Kit is tested for isolation of DNA from E.coli. The quantity and quality of purified DNA attend to:
• Ratio 260/ 280.
• Agarose gel electrophoresis.
• Digestion with restriction endonucleases

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5. Kit Storage

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K and Lysozyme at 4°C, RNase A solution at -20°C, all other kit components can be stored at room temperature.

6. Applications

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

7. 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.

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DETAILED PROTOCOL

1. Pour the culture in a 1.5 ml centrifuge tube and Harvest the bacterial cells by centrifugation at 13000 rpm for 1 minute. Discard supernatant.

2. Resuspend the cell pellet in 180 µl of Buffer Solution BR-1

3. Add 10 µl of Lysozyme and incubate 30 minute at 37°C.

4. [Optional]. If RNA-free genomic DNA is required, add 20 µl RNase A solution, mix, and incubate for 10 minutes at 37°C.

GRAM NEGATIVE BACTERIA

5. Add 20 µl of Proteinase K and incubate 1 hour at 55°C. (vortex occasionally during the incubation).

6. Add 200 µl of Buffer BLU, vortex and incubate 10 minutes at 70°C.

GRAM POSITIVE BACTERIA

5. Add 25 µl of Proteinase K and 200 µl of buffer BLU. Vortex.

Do not add proteinase K directly to Buffer BLU.

6. Incubate 30 minutes at 70°C.

7. Add 200 µl of ethanol (96–100%) (not provided) and mix by vortexing vigorously.

8. Transfer the mix to the minispin column by pipetting and centrifuge at 13000 rpm for 1 minute. Discard

the flow-through

9. Place the minispin column in a collection tube and add 500 µL of WB1 buffer. Centrifuge at 13000 rpm

for 1 minute. Discard the flow-through

10. Place the minispin column in a collection tube and add 500 µL of WB2 buffer. Centrifuge at 13000 rpm

for 3 minute. Discard the flow-through.

11. Place the minispin column into a new, labelled 1.5 microcentrifuge tube and pipet 100µL EB BuffeR directly into the membrane or pre-warm water. Close the cap and incubate for 1 minute at room temperature.

12. Centrifuge at 13000 rpm for 1 minute elute DNA