Data sheet

Bacteria Genomic DNA Isolation Kit

Cat. No: AN0066(50 reactions)
Cat. No: AN0067 (100 reactions)

Description

Bacteria 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

Kit Components

	AN0066	AN0067
Minispin columns	50	100
Collection tubes (2 mL)	50	100
BR-1 Buffer	15 ml	30 ml
BLU Buffer	20 ml	40 ml
WB1 Buffer	30 ml	60 ml
WB2 Buffer*	6 ml	2X6 ml
EB buffer	15 ml	30 ml

- Digestion with restriction enzymes.
- Automated sequencing.
- PCR template.
- Southern Blots.

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

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.

Kit Storage:

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K and Lysozyme at 4ºC, RNase A solution at -20ºC, all other kit components can be stored at room temperature. The kit components are stable for 1 year, if stored properly.

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

GRAM NEGATIVE BACTERIA

5. Add 20 µl of Proteinase K and incubate 1 hour at 55ºC. (vortex occasionally during the incubation).
6. Add 200 µl of Buffer BLU, vortex and incubate 10 minutes at 70ºC.

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 70ºC.

7. Add 200 µl of ethanol (96–100%) 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

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.