dNEAT™ Bacterial Genomic DNA Isolation Kit

PURK-BAC-050

Description

This 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.

Applications

All molecular biology applications such as:

- ✓ 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

Kit Components	50 rxn
Minispin columns	50
Collection tubes (2 ml)	50
BR-1 Buffer	15 ml
BLU Buffer	20 ml
WB1 Buffer	30 ml
WB2 Buffer*	6 ml
EB Buffer	15 ml
Proteinase K**	30 mg
Lysozime***	25 mg
RNase A Solution (10 mg/ml)	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.

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.

Product use limitation

This product is developed and sold exclusively for research purposes only. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

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 minutes 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 minutes. 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 and elute DNA