KIAGENE FANAVAR

Bactokia
Bacterial DNA Isolation kit

Cat. No:

FPKT024.0025
FPKT024.0050
FPKT024.0100

Contents:

Components	25 preps	50 preps	100 preps
BB1 Buffer (blue)	8 ml	15 ml	30 ml
BB2 Buffer (orange)	8 ml	15 ml	30 ml
BB3 Buffer (yellow)	15 ml	25 ml	50 ml
BB4 Buffer (green)	4 ml	8 ml	16 ml
PEB Buffer (white)	2 ml	2 ml	2 ml
Proteinase K 20mg/ml	50 µl	100 µl	200 µl
RNase A 10mg/ml	50 µl	100 µl	200 µl
Lysozyme 20mg/ml	0.5 ml	1 ml	2 ml
Column and collection tubes	25 each	50 each	100 each

Kit storage:

⚠ This kit should be stored at room temperature.

⚠ **Proteinase K, Lysozyme** and **RNase A** should be stored at -20 °C

If properly stored, all kit components are stable until the expiration date printed on the label.

⚠ After adding **RNase A** to **BB1 buffer** and **absolute ethanol** to **BB4**, Store the **BB1** and **BB4** at +2 to +8°C

Additional Equipment and Reagent required

•Absolute ethanol

•Standard tabletop microcentrifuge capable of 13,000 xg centrifugal force

•Microcentrifuge tubes, 1.5 ml, sterile

Application

Bactokia Kit provides a simple and convenient technique to isolate high quality DNA from both Gram-negative and Gram-positive bacteria. This kit combines the advantages of a silica-based system with a microspin format, eliminating the need for expensive resins and hazardous organic compounds. Bacteria are first incubated with the appropriate enzymes to ensure efficient cell lysis and DNA release from the cells and is fully compatible with all downstream applications such as PCR, qPCR, NGS and microarrays since all humic acid substances and other PCR inhibitors are removed during the isolation process. The procedure is optimized to achieve reliable results within **60 min**.

Handling Requirements and Safety Information

⚠ *All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.*

⚠ *BB3 Buffer contain guanidinium hydrochloride which is an irritant.*

⚠ *Do not allow BB2 Buffer and BB3 Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.*

⚠ *If you spill the reagent, dilute the spill with water before wiping it up.*

⚠ *Do not use any modified ethanol.*

⚠ *Do not pool reagents from different lot numbers.*

⚠ *Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.*

⚠ *After first opening store all bottles in an upright position.*

⚠ *Do not allow the BB2 Buffer and BB3 Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.*

⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

preparation procedure:

Working Solution Preparation

⚠ **Add RNase A to BB1 Buffer completely Dissolve the RNase A by vortexing. store the BB1 Buffer at 4 °C.**

FPKT024.0025-25 preps

⚠ **Add 16 ml absolute ethanol** to **BB4 Buffer** Bottle.

FPKT024.0050-50 preps

⚠ **Add 32 ml absolute ethanol** to **BB4 Buffer** Bottle.

FPKT024.0100-100 preps

⚠ **Add 64 ml absolute ethanol** to **BB4 Buffer** Bottle.

Please note that the Ethanol concentration of a BB4 Buffer may decrease during long term storage resulting in a drop-down of the final DNA yield.

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⚠ before starting Incubate the **PEB Buffer at 55 to 65 ° C** until the end of the protocol to obtain the maximum yields.

Protocol

1. Sample preparation
Pellet the bacterial cells from 1-4 ml of bacterial culture by centrifuge in 8000-10000 g for 1 min. Discard the supernatant

2. Add 200 µl **BB1 Buffer** to the bacterial pellet. Vortex at maximum speed for 30 Secound.

3. Add 15 µl **Lysozyme** to the sample microtube and incubate at 37° C for 30 minutes. Invert the tube every 5 minutes.

4. Add 200 µl **BB2 Buffer** to the microtube and mix them by Vortex.

5. Add 2.5 µl of **Proteinase K** then mix by vortex. Incubate at 60 °C for at least 15 minutes. During incubation, invert the tube every 3 minutes.

6. Add 400 µl of **BB3 Buffer** to the sample and mix by vortex for 10 seconds. Briefly spin the tube to remove drops from the inside of the lid. Centrifuge at full speed (~18,000 x g) for 1 minutes.

7. Transfer the supernatant into a **DNA spin column** assembled in a clean collection tube (provided). Centrifuge at 10000 g for 1 min at room temperature.

8. Disconnect the **DNA spin column** from collection tube and discard the flow through solution. Reconnect the DNA spin column to the collection tube.

9. Add 500 µl **BB4 Buffer** to DNA spin column and centrifuge at 10000 g for 1 min. Discard flow through.

10. Centrifuge the **DNA spin column** at 10000 g for 1 min to remove residual ethanol.

11. Place the DNA spin column into the clean new microtube. Add 30 µl of pre-warmed **PEB Buffer** directly onto column membrane and stand for 3 min. Centrifuge at 10000 g for 1 min.

12. Add another 30 µl pre-warmed **PEB Buffer** or sterile DDW directly onto column membrane and stand for 3 min. Centrifuge at 10000 g for 1 min.

13. Check 7-10 µl of extracted DNA by 1% agarose gel electrophoresis. Store DNA at -20˚C