

ab288102 – Bacterial Genomic DNA Isolation Kit

For isolating quality genomic DNA from gram-negative and gram-positive bacterial species.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab288102>

Storage and Stability

Store kit at -20°C and room temperature (RT), protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Buffer A [Re-suspension Buffer]	25 ml	RT
Enzyme Mix A	1 ml	-20°C
RNAse A	600 µl	-20°C
Buffer B [Reaction Buffer]	1.7 ml	RT
Enzyme Mix B	1.2 ml	-20°C
Buffer C [Binding Buffer]	25 ml	RT
Buffer D [Wash Buffer]	30 ml	RT
Buffer E [Elution Buffer]	22 ml	RT
Spin Columns/Collection Tubes	100 tubes	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- DNAse-free aerosol tips and micro-centrifuge tubes
- 100% Ethanol
- Heating Block
- Centrifuge
- Mutanolysin and Lysostaphin.

Reagent Preparation

- Add 28 mL 100% Ethanol to the Buffer C [Binding Buffer] bottle and 136 mL 100% Ethanol to the Buffer D [Wash Buffer] bottle before use.
- Briefly centrifuge small vials prior to opening.
- Read entire protocol before performing the assay.

Buffer A, B, and E: Ready to use. Store at RT.

Buffer C: Add 28 ml of 100% Ethanol, molecular biology grade. Mix well and store at RT.

Buffer D: Add 136 ml of 100% Ethanol, molecular biology grade. Mix well and store at RT.

Enzyme Mix A, Enzyme Mix B, and RNAseA: Ready to use. Store at -20°C. Keep on ice at all times while in use.

Spin Columns: Ready to use. Store at RT in dry conditions.

Assay Protocol

1. Transfer 1ml of overnight bacterial culture into a 1.5 ml tube and pellet the cells by centrifugation at 12,000 x g for 1 min at 4°C. Discard supernatant.

Δ Note: If using >1-1.5 ml overnight culture, scale up the entire protocol proportionally.

2. Re-suspend the pellet in 250 µl Buffer A [Re-suspension Buffer].
3. Add 10 µl of Enzyme Mix A. If RNA free DNA is desired, add 6 µl of RNAse A solution at this time. Mix by inverting the tube 3-5 times.

Δ Note: Certain bacterial species require additional enzymes during cell lysis. For *Streptococcus mutans* and *Staphylococcus* species, supplement the lysis reaction with ≥ 250 units/mL *Mutanolysin** and ≥ 200 units/mL *Lysostaphin** respectively. *Not supplied.

4. Incubate for 30 min at 37°C.
5. Add 17 µl of Buffer B [Reaction Buffer] and 12 µl of Enzyme Mix B. Mix by inverting the tube 3-5 times.
6. Incubate for 30 min at 55°C.
7. Add 500 µl of Buffer C [Binding Buffer] and mix by pipetting up and down until the solution becomes clear.
8. Place the provided spin column into the provided collection tube and pipette the entire supernatant onto the top of the column.
9. Spin at 12,000 x g for 1 min at 4°C and discard the flow through.
10. Add 750 µl of Buffer D [Wash Buffer] onto the top of the spin column and spin (12,000 x g; 1 min., 4°C). Discard the flow through.
11. Repeat step 10 one more time.
12. Spin the spin column at 12,000 x g for 2 min at 4°C to dry.
13. Transfer the spin column to a clean, DNAse-free 1.5ml tube.
14. Add 200 µl of Buffer E [Elution Buffer] to the top of the spin column and incubate for 1-2 min at RT.
15. Spin at 12,000 x g for 1 min at 4°C to elute the genomic DNA.
16. Sample is now purified and ready to use. Store genomic DNA at -20°C or immediately use the sample in a downstream application of your choice.

Δ Note: Generally, good quality genomic DNA will have A260/280 of 1.7- 1.99 and exhibit one clear band of high molecular weight on 1% agarose gel. See technical support for help.

Technical Support

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