PRODUCT: **Bactozol™ Enzyme Solution** August 2023
Cat. No: BZ 160

PRODUCT DESCRIPTION

Bactozol™ Enzyme Solution contains activated lysozyme that effectively lyses a broad range of gram-negative and gram-positive bacteria. Bactozol Enzyme Solution is supplied as a convenient 10X stock solution that maintains enzyme stability at room temperature and precludes the daily preparation of an enzyme stock solution. The 10X Bactozol Enzyme Solution is diluted with Bactozol Enzyme Dilution Buffer prior to bacterial digestion. After bacterial lysis with 1X Bactozol Enzyme Solution, DNA can be isolated from the lysate with DNAzol® (1) or other suitable DNA isolation procedures (Note 1). The Bactozol Enzyme Solution protocol produces a high quality bacterial genomic and plasmid DNA lysate with a simple and efficient protocol. Sufficient Bactozol Enzyme Solution is provided to prepare 125 lysates, each yielding up to 40 µg of bacterial DNA.

STORAGE AND STABILITY: 10X Bactozol Enzyme Solution is stable for six months when stored at room temperature, for at least one year when stored at 4 - 8 C and for longer periods when stored at -20 C. Always store Bactozol Enzyme Solution as a 10X stock solution; use 1X Bactozol Enzyme Solution within 24 hours following dilution. Bactozol Enzyme Dilution Buffer is stable for two years when stored at room temperature.

HANDLING PRECAUTIONS: Bactozol Enzyme Solution contains irritants. Refer to the Bactozol Enzyme Solution SDS for additional information (www.mrcgene.com). All chemicals may pose unknown hazards and should be used with caution by following good laboratory practice.

PROTOCOL

Reagents supplied: 10X Bactozol Enzyme Solution and Bactozol Enzyme Dilution Buffer. This bacterial lysing protocol describes processing of samples derived from 0.5 - 2 ml of culture containing up to 40 µg of bacterial DNA. The protocol can be scaled up or down to isolate DNA from larger or smaller bacterial samples. For some gram-positive bacteria that are resistant to lysis, we recommend an extended lysis procedure or the inclusion of additional enzymes appropriate for specific bacterial strains (Notes 2, 3).

1. LYSIS

Dilute an aliquot of 10X Bactozol Enzyme Solution with nine volumes of the Bactozol Enzyme Dilution Buffer to obtain the required amount of 1X Bactozol Enzyme Solution. 1X Bactozol Enzyme Solution should be used within 24 h. Sediment 0.5 - 2.0 ml of bacterial suspension at 6,000 g for 4 minutes at 4 - 25 C. Discard the supernatant and resuspend the bacterial pellet in 100 ul of 1X Bactozol Enzyme Solution by vortexing or pipetting to achieve a homogenous suspension. For samples that contain large quantities of exopolysaccharide mucus, pipette to resuspend the bacterial pellet in the 1X Bactozol Enzyme Solution. Incubate the bacterial suspension at 50 C for 15 - 30 minutes for gram-negative and 20 - 60 minutes for gram-positive bacteria (Notes 2, 3).

2. BACTERIAL LYSATE

Persistent turbidity following bacterial lysis may be due to insoluble cell wall debris (Note 4). After lysing the bacterial cell wall, bacterial DNA can be isolated and recovered with DNAzol® (1) or other suitable DNA extraction procedures.

NOTES

1. The Bactozol Kit (Cat. no. BA 154) provides both Bactozol Enzyme Solution and DNAzol® in one fast, efficient and economical procedure to lyse bacteria and isolate bacterial DNA.
2. Persistent cloudiness of the 1X Bactozol Enzyme Solution lysate may indicate incomplete lysis. To improve cell lysis and DNA recovery, extend the incubation time an additional 15 - 30 minutes and elevate the incubation temperature to 55 C prior to DNA isolation using DNAzol® or another suitable DNA extraction procedure.
3. Bactozol Enzyme Solution provides excellent lysis with a wide array of bacterial strains. Some gram-positive bacteria, such as Staphylococcus and Streptococcus, are more difficult to lyse. To improve bacterial lysis in these recalcitrant strains, pretreat the bacterial suspensions with specific enzymes that are known to enhance lysis in that strain. See MRC Technical Bulletin 8 for additional information.
4. If the bacterial lysate remains turbid at the end of the digestion, it may contain insoluble cell wall debris. Centrifuge the lysate at 10,000 g for 7 minutes at 4 - 25 C, collect the supernatant and continue the procedure with the DNA isolation.

REFERENCES

1. Chomczynski P, Mackey K, Drews R and Wilfinger W. (1997) DNAzol: A reagent for the rapid isolation of genomic DNA. BioTechniques 22, 550-553.

Bactozol™ Enzyme Solution, Manufacturer protocol, 2023. Molecular Research Center, Inc., Cincinnati, OH.