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EXTENDED BACTOZOL ENZYME SOLUTION™ LYSIS PROCEDURE FOR THE ISOLATION OF GENOMIC DNA FROM RECALCITRANT GRAM-POSITIVE BACTERIA

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Bactozol Enzyme Solution™ is a stable enzymatic solution containing activated lysozyme that effectively lyses a broad range of gram-negative and gram-positive bacteria. Some gram-positive bacteria, such as Staphylococcus and Streptococcus, are resistant to Bactozol Enzyme Solution cell wall lysis and additional enzymes are required to maximize DNA recovery from these cells. A variety of lysing protocols have been developed for Streptococcal (1) and Staphylococcal (2) bacterial preparations. Most of these use enzymes such as proteinase K, mutanolysin or lysostaphin to penetrate the exopolysaccharide biofilm and augment digestion of the cell wall. When Streptococcal and Staphylococcal cells are pretreated with additional enzymes in conjunction with Bactozol Enzyme Solution digestion, DNA yields can be significantly improved.

MODIFIED BACTOZOL ENZYME SOLUTION LYSIS PROTOCOL

Obtain a bacterial pellet by sedimenting 0.5 - 2.0 ml of bacterial suspension at 6,000 g for 4 minutes at 4 - 25 C. Decant the supernatant and resuspend the bacterial pellet in 0.1 ml of the designated enzyme mixture as described below. Incubate for 60 - 90 minutes at 45 C. Keep the bacteria suspended in the lysing solution with periodic mixing. At the end of this first incubation, add 10 µL of 10X Bactozol Enzyme Solution, mix with gentle vortexing and incubate for an additional 30 - 60 minutes at 45 C. At the end of the second incubation, mix the sample with a pipette (25 X) before adding 0.4 ml of DNAzol® and 4 µl of MRC Polyacryl Carrier (Cat. No. PC 152) to the lysate. Incubate the DNAzol/lysate mixture at 45 C for an additional 15 minutes and process the sample as described in the Bactozol Kit Bacterial DNA Isolation protocol (Steps 3 - 6).

ALTERNATIVE ENZYME STOCK SOLUTIONS FOR RESPECTIVE BACTERIAL STRAINS

Proteinase K (Sigma P-2308): Dissolve the enzyme in distilled water to a final concentration of 20 mg/ml. Prepare 50 - 100 µl aliquots and store the enzyme at -20 C. Use 2 - 5 µl of proteinase K stock solution (40 - 100 µg/0.1ml) per sample.

Mutanolysin (Sigma M-9901) for Streptococcus: Resuspend the lyophilized enzyme in distilled water to a final concentration of 25,000 U/ml. Prepare 50 - 200 µL aliquots and store the enzyme at -20 C. Use 1 - 2 µl of mutanolysin stock solution (25 - 50 U/0.1ml) per sample. Resuspend the Streptococcal cells in a 0.1 ml freshly prepared mixture of proteinase K and mutanolysin (e.g., 5 µl of proteinase K + 2 µl of mutanolysin + 93 µl Bactozol Enzyme Dilution Buffer; see Note).

Lysostaphin (Sigma L-0761) for Staphylococcus: 4000 U/ml in 20mM sodium acetate, stored at -20C. Use 1 - 5 µl of lysostaphin (4 - 20 U/0.1 ml) per sample. Resuspend the Staphylococcal cells in a 0.1 ml freshly prepared mixture of proteinase K and lysostaphin (e.g., 5 µl of proteinase K + 5 µl of lysostaphin + 90 µL Bactozol Enzyme Dilution Buffer; see Note).

NOTES

DNA recovery may be improved in some preparations by replacing the Bactozol Enzyme Dilution Buffer with a high salt solution containing 2.5 M NaCl, 50 mM EDTA, pH 7.5. However, RNA degradation is inhibited in the presence of high salt and RNA contamination will be significant.

REFERENCES

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2. Johnson, AP and Woodford, N. (1998) Plasmid Analysis. Methods in Molecular Medicine, Vol. 15: Molecular Bacteriology: Protocols and Clinical Applications; edited by: N. Woodford and AP Johnson, Humana Press Inc., Totowa, NJ. 51-62.

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