PRODUCT: **BACTOZOL™ Bacterial DNA Isolation Kit** June 2014
Cat. No: BA 154

PRODUCT DESCRIPTION

Bactozol™ is a kit for the isolation of DNA from gram-negative and gram-positive bacteria. The kit includes three reagents, 10X Bactozol Enzyme Solution, Bactozol Enzyme Dilution Buffer and DNAzol®. Bactozol Enzyme Solution contains activated lysozyme for the effective lysis of a broad range of bacterial specimens. It is supplied as a convenient 10X stock solution that maintains enzyme stability at room temperature and precludes the daily preparation of lysozyme stock solutions. After bacterial lysis with 1X Bactozol Enzyme Solution, DNA is isolated from the lysate with DNAzol (1). The Bactozol Kit protocol produces high quality bacterial genomic and plasmid DNA with a simple and efficient protocol. The kit includes sufficient reagents for 125 isolation procedures, each yielding up to 40 µg of 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. DNAzol and Bactozol Enzyme Dilution Buffer are stable for two years when stored at room temperature.

HANDLING PRECAUTIONS: DNAzol and 10X Bactozol Enzyme Solution contain irritants. Refer to the DNAzol and 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, Bactozol Enzyme Dilution Buffer and DNAzol. Reagents required but not supplied: ethanol, Polyacryl Carrier (PC 152) when expected yield is < 5 µg DNA.
This 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 gram-positive bacteria resistant to lysis, we recommend an extended lysis procedure or the inclusion of additional enzymes appropriate for specific bacterial strains (see Notes 1 and 2).

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1. LYSIS	- bacterial pellet + 100 µl 1X Bactozol Enzyme Solution. Store 15 - 30 min at 50 C.
2. LYSATE SOLUBILIZATION	- lysate + 0.4 ml DNAzol. Store 5 - 15 min.
3. DNA PRECIPITATION	- DNAzol - lysate solution + 0.3 ml ethanol. 3,000 g x 4 min.
4. DNA WASH	- 1 ml 75% ethanol.
5. DNA SOLUBILIZATION	- TE buffer, water or 8 mM NaOH.

The procedure is performed at room temperature unless stated otherwise.

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 a bacterial suspension at 6,000 g for 4 minutes at 4 - 25 C. Discard the supernatant and resuspend the bacterial pellet in 100 µl of 1X Bactozol Enzyme Solution by vortexing to achieve a homogenous suspension. For samples that contain large quantities of exopolysaccharide mucus, pipet the pellet to resuspend the bacteria in the 1X Bactozol Enzyme Solution. Lyse the bacterial suspension by incubating at 50 C for 15 - 30 minutes for gram-negative and 20 - 60 minutes for gram-positive bacteria (Notes 1, 2).

2. LYSATE SOLUBILIZATION

Mix the resulting bacterial lysate (0.1 ml) with four volumes of DNAzol (0.4 ml) by hand for 15 - 30 seconds to obtain a homogeneous solution. If expected yield is < 5 µg DNA, add 2 - 5 µl of Polyacryl Carrier to the DNAzol - lysate solution and mix. (Note 3). Incubate the lysate for 5 minutes at room temperature. When processing large samples or cloudy bacterial lysates, extend the DNAzol incubation to 15 minutes and increase the temperature to 50 C to improve DNA quality and recovery. Avoid excessive vortexing to minimize DNA shearing (Note 4).

3. DNA PRECIPITATION

Precipitate the DNA from the DNAzol - lysate solution (0.5 ml) by adding 0.6 volumes of 100% ethanol (0.3 ml). Mix the lysate by inversion for 15 seconds and store at room temperature for 2 - 5 minutes. Samples containing > 10 µg of bacterial DNA often form a white, thread-like precipitate. Sediment the DNA by centrifugation at 3,000 g for 4 min at 4 - 25 C (Note 5).

4. DNA WASH

Following centrifugation, carefully decant the supernatant and remove the residual fluid with a pipet. Wash the DNA pellet with 1 - 1.5 ml of 75% ethanol by vortexing. Store the sample for 1 - 2 minutes to allow the pellet to sediment and drain the ethanol by decanting. Store the tube inverted for 1 - 2 minutes and remove any residual ethanol with a pipet. A second wash step with 75% ethanol may improve DNA quality in some preparations. Remove the residual ethanol and solubilize the DNA pellet while it is still wet. Drying the DNA pellet is not recommended since it will impede DNA solubilization.

Typically, this washing step can be performed without centrifugation. However, small amounts of DNA (< 5 µg) can be more efficiently recovered by precipitation in the presence of Polyacryl Carrier (Note 3) and centrifugation at 3,000 g for 4 minutes at 4 - 25 C.

5. DNA SOLUBILIZATION

Add 50 - 300 µl of TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7 - 8) or water to the DNA pellet. Rehydrate the DNA pellet for 10 - 15 minutes at room temperature or 50 C and mix or pipette the sample until the gelatinous DNA is completely dissolved. Alternatively, solubilize the DNA pellet in 8 mM NaOH. Following solubilization in NaOH, neutralize the DNA solution with HEPES as described in the DNAzol protocol (Step 5; DNAzol Protocol; www.mrcgene.com). Add an adequate volume of TE, water or NaOH to approach a DNA concentration of 0.1 - 0.2 µg/µl. If necessary, remove any residual insoluble debris from the solubilized DNA by centrifugation at 10,000 g for 7 minutes at 4 - 25 C.

6. QUANTITATION OF DNA AND RESULTS

Determine the A_{260} and A_{280} of the solution and calculate the DNA content assuming that one A_{260} unit equals 50 g of double stranded DNA/ml. The $A_{260/280}$ ratio of the isolated DNA is within the 1.6 - 1.8 range. A low $A_{260/280}$ ratio may be due to incompletely solubilized DNA. If the DNA concentration is > 0.2 µg/µl, further dilution and mixing may improve the sample $A_{260/280}$ ratio. For optimal spectrophotometric measurements, the DNA aliquot should be diluted with water or a buffer with a pH >7.5. Distilled water with a pH < 7 artificially decreases the $A_{260/280}$ ratio of nucleic acids (2).

Typically, ethidium bromide staining of DNA separated in a 1% agarose gel visualizes high molecular weight DNA near the application slot and a second DNA band in the 20 - 40 kb range. The isolated DNA is suitable for PCR, Southern blotting and other molecular biology applications. DNA smearing from the loading slot to the 20 - 40 kb band is usually due to incomplete DNA solubilization. Dilute and re-mix the sample for optimal separation.

NOTES

1. Persistent cloudiness of the lysate may indicate incomplete bacterial 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 before adding DNAzol to the sample.
2. Some gram-positive bacteria, such as Staphylococcus and Streptococcus, are more difficult to lyse and often produce lower DNA recovery. To improve bacterial lysis and DNA recovery, pretreat the bacterial suspensions with specific enzymes that are known to enhance lysis in that strain. See Technical Bulletin 8 for additional information.
3. The efficiency of DNA recovery from a bacterial sample containing small quantities of DNA (< 5µg) is improved by the addition of Polyacryl Carrier (cat. no. PC 152). Add 2 - 5 µl of the carrier to the DNAzol - lysate solution (Step 2) and proceed with the ethanol precipitation of DNA according to protocol.
4. If the DNAzol-lysate is turbid, it may contain insoluble cell wall debris that makes it more difficult to resolubilize the isolated DNA. Centrifuge the DNAzol - lysate at 10,000 g for 7 minutes at 4 - 25 C, collect the supernatant and precipitate the DNA according to Step 3 of the protocol.
5. In samples containing > 10 µg of DNA, ethanol precipitation (Step 3) may yield a large DNA precipitate that floats in the DNAzol - lysate solution. Use a pipet tip to spool the DNA precipitate and transfer it to a clean tube. After removing residual DNAzol from the DNA precipitate, wash the precipitate with 75% ethanol according to the protocol (Step 4). Omit centrifugation and decant the ethanol wash solution. By collecting the spooled DNA with a pipet as described here, DNA can be isolated without the use of a centrifuge.

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.
2. Wilfinger, W., Mackey, K. and Chomczynski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques, 22, 474-481.

Bactozol™ Manufacturer protocol, 2014. Molecular Research Center, Inc., Cincinnati, OH.