PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Please visit www.biobasic.com

A world Leader in Serving Science

A world leader in serving science

BIO BASIC INC.

EZ-DNA Reagents

BS8202

Version 5.1
ISO9001 Certified

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

EZ-DNA Reagents

Product information for BS8202:

Kit Contents

Components	BS8202, 100 Preps
EZ-DNA Reagent	100 ml
Protocol	1

Storage and Stability

EZ-DNA Reagent is stable at 15 to 30 °C for at least one year after the date of purchase.

Safety Instructions

EZ-DNA Reagent is harmful in contact with skin or if swallowed; please avoid contact with eyes, skin, and clothes. Wash thoroughly after handling and see doctor if necessary. Keep container closed.

Introduction

EZ DNA Reagent is a ready-to-use solution for isolation of genomic DNA from various samples of

Note1: Weak alkaline solutions are neutralized by CO_2 from the air. Make fresh 8 mM NaOH every month from a 2-4 M NaOH stock solution prepared within six months.

Note2: The isolated DNA contains partially degraded RNA. If a reduction of the RNA content to less than 3% is necessary, perform the centrifugation step as described in Step 2 of the protocol. In Southern analysis, RNA can be digested by supplementing of the restriction mix with RNase A (1 µg/ml).

DNA to settle to the bottom of the tubes and remove ethanol carefully by pipetting or decanting.

5. DNA Solubilization.

A. Air dry the DNA by storing in an open tube for 5-15 seconds after removing the ethanol.

> Note: If the DNA is exposed to air for more than a few seconds, it will be much more difficult to dissolve.

B. Dissolve the DNA in 8 mM NaOH by slowly passing the pellet through a pipette tip. Use of the 8 mM NaOH assures full soluble of the DNA precipitate. Add an adequate amount of the 8 mM NaOH to approach a DNA concentration of 0.2-0.3 µg/µl. Typically add 0.2-0.3 ml of 8 mM NaOH to the DNA isolated from 10^7 cells or 10-20 mg of animal tissue.

> Note: DNA will not be fully soluble in TE or water. The resolubilization of EZ-DNA isolated DNA is low in Tris buffers. Therefore the use of 8 mM NaOH is highly recommended. DNA is stable in 8 mM NaOH for several months at 4 °C and greater than one year at -20 °C.

C. The DNA preparations isolated from tissues such as liver, muscles, and plants may contain some insoluble materials (mostly polysaccharides). Remove the insoluble materials by centrifugation at 12,000 × *g* for 10 min.

animal tissue, cultured cells, plant, yeast or bacteria. EZ-DNA Reagent method is based on the use of an optimized guanidine-detergent solution. During the isolation procedure, tissue or cells are lysed in EZ-DNA Reagent and the genomic DNA is precipitated from the lysate in the presence of ethanol. Following an ethanol wash, purified DNA is recovered in water. Isolated DNA can be used directly for most applications such as Southern analysis, Molecular cloning and/or PCR.

Features

1. Fast and Effective. Entire procedure takes 10-30 minutes with DNA recovery of 70~90%.
2. Batch Isolation. EZ DNA Regeant is suitable for batch isolation. Multiple samples can be processed simultaneously.
3. No phenol/chloroform steps required.

Reagents and Materials Supplied by User:

Microcentrifuge capable of at least 12,000 × *g*
Sterile 1.5 ml or 2 ml Centrifuge Tubes
Lysozyme solution (3 mg/ml)
Absolute (96%-100%) Ethanol
8 mM NaOH (freshly prepared)

Protocol

1. Sample Preparation.

A. Animal tissues: Homogenize 25-50 mg tissue in 1 ml of EZ-DNA Reagent using a pestle or

homogenizer. Alternatively, grind the tissue to fine powder in liquid nitrogen or dry ice/ethanol before extraction with EZ-DNA Reagent.

B. Adherent Cell culture: Add 0.75-1.0 ml of EZ-DNA Reagent per 10 cm^2 culture plate area. Lyse the cells by agitating the culture plate and gently pipet the lysate into an assay tube.

C. Suspensions cell culture: Add 1 ml of EZ-DNA Reagent to 1-3 × 10^7 cells, either in pellet or in suspension (volume < 0.1 ml). Lyse the cells by gently pipetting.

D. Gram-negative bacteria: Transfer 1ml overnight culture (about 2 x 10^9 cells) into a 1.5 ml centrifuge tube and centrifuge at 8,000 x *g* for 30 seconds, discard supernatant. Add 1 ml EZ-DNA to pellet, vortex well, and lyse cells at room temperature for 5-10 min.

E. Gram-positive bacteria: Transfer 1 ml overnight culture (about 2 x 10^9 cells) into a centrifuge tube and centrifuge at 10,000 x *g* for 30 seconds, discard supernatant. Add 180 µl lysozyme solution (3 mg/ml) to the pellet, after 30-60 min of digestion at 37 °C, add 820 µl EZ-DNA, and lysis cells at room temperature for 5-10 min

> **Note:** To minimize shearing of the DNA molecules, pipet DNA solution using wide-mouth pipette tips. Prepare wide bore pipette tips by cutting 2-3 mm from the ends of plastic pipette tips. Mix DNA solutions by gentle inversion.

2. Centrifugation (optional).

Centrifuge the homogenate for 10 min at 10,000 × *g* at 4 °C or room temperature. Transfer supernatant to a fresh tube.

> **Note:** The supernatant may appear viscous. This step removes insoluble tissue fragments, RNA, and excess polysaccharides from the lysate/homogenate. This step is only required for the isolation of DNA from tissues such as liver, muscles, as well as most plant tissues which contain a large amount of cellular and/or extracellular material. This process is recommended to minimize RNA carry-over into the DNA.

3. DNA Precipitation.

Add 0.5 ml of ice-cold 100% ethanol per 1 ml of EZ-DNA Reagent used for the isolation. Mix samples by inversion them several times. DNA should quickly become visible as a cloudy precipitation. Centrifuge the mixture for 5-10 min at 10,000 × *g* at 4 °C. Decant the supernatant carefully. Place the tubes upright for 1 min and aspirate the remaining lysate from the bottom of the tubes.

4. DNA Wash.

Wash the DNA precipitate twice with 0.8-1.0 ml of 75% ethanol. Centrifuge the mixture for 5-10 min at 10,000 × *g* at 4 °C. At each wash, re-suspend the pellet in ethanol by inverting the tubes 3-6 times. Store the tubes vertically for 0.5-1 min to allow the