Ver. 23081

ODP320

Prestozol Reagent

For Isolation of Genomic DNA from solid and liquid samples of animal, yeast, and bacterial origin.

Origin

ISO 13485:2016 ISO 9001:2015

Prestozol Reagent

(Cat.No.: ODP320)

Introduction

Prestozol Reagent is a ready-to-use solution for the isolation of genomic DNA from solid and liquid samples of animal, yeast, and bacterial origin. Prestozol Reagent procedure can be completed in typically 10–30 minutes with DNA recoveries of 60–90%.

The Prestozol Reagent procedure is based on the use of a proprietary lysing solution which permits selective precipitation of DNA from cell/tissue lysate. The isolated DNA can be used without additional purification for applications such as Southern analysis, dot blot hybridization, molecular cloning, and polymerase chain reaction (PCR).

Product contents

Contents	Volume
Prestozol Reagent	100mL

Storage

- Prestozol Reagent can be stored at 15°C to 30°C for up to 12 months without showing any reduction in performance and quality.

Reagents required, but not supplied

- 99-100% Ice-cold Isopropanol
- 75% Ethanol
- 8mM NaOH

Protocol

Summary of the protocol:

1.	Lysis/Homogenization	25-50mg tissue/ 1-3 × 10^7 cells/ 0.1mL liquid sample per 1mL Prestozol Reagent
2.	Centrifugation	12,000 rpm, 10 minutes
3.	DNA Precipitation	Lysate + 0.5mL 100% ice-cold isopropanol
4.	DNA Wash	0.5mL 75% ethanol
5.	DNA Solubilization	8mM NaOH

This procedure should be carried out at room temperature

1. Preparation of samples
   a) Cells grown in monolayer: Add 0.75-1.0mL of Prestozol Reagent per 10cm^2 culture plate area. Lyse the cells by agitating the culture plate and gently pipette the lysate into an assay tube.
   b) Cell Pellets or Suspensions: Add 1mL of Prestozol Reagent to 1-3 × 10^7 cells, either in pellet or in suspension (volume < 0.1mL). Lyse the cells by gentle pipetting.
   c) Yeast/fungal samples: Add 600µL Buffer LY (Cat.No.: ORT409, not provided), 100µL β-Mercaptoethanol and 10µL Lyticase (Cat.No.: ORT410, not provided) to 1-5 ×10^9 yeast cells or 100 mg of mycelium. Incubate the sample at room temperature for 30 minutes. Occasionally mix the content by inverting the tube. The volume of Buffer LY, Lyticase and β-Mercaptoethanol will vary with cell number and type of cells. Centrifuge the sample at 4,000 rpm for 5 minutes and remove the supernatant. Add 1mL of Prestozol Reagent to the pellet.
   d) Bacterial samples: Pipette 1-5mL overnight bacterial culture suspension in a microcentrifuge tube by centrifuging for 1 minute at 10,000 rpm. Discard supernatant.
      - Gram negative cells: Add 1mL Prestozol Reagent and mix by gently pipetting.
      - Gram positive cells: Add 180µL Buffer LZ (Cat.No.: ORT402, not provided) and 20µL Lysozyme (Cat.No.: ORT401, not provided). Incubate for at least 30 minutes at 37°C. Add 30mg glass beads (1mm) (Cat.No.: OWL045, not provided) and 1mL Prestozol Reagent to the sample. Vortex for 15-30 seconds and incubate at 80°C for 10 minutes.
   e) Whole blood: For whole blood up to 200µL, add 1mL of Prestozol to the blood and pipette up and down gently to lyse the cells. For sample volumes >200µL, e.g. 300µL - 1mL, add 3 times volume Red Cell Lysis Buffer (Cat.No.: ORT122, not provided) to the sample, close the cap and invert the tube. Place the tube in room temperature (15–25°C) for 5 minutes, and centrifuge at 12,000 rpm for 1 minute, then discard the supernatant and pipette 1mL Prestozol Reagent and mix by pulse-vortexing.
   f) Cell Nuclei: Add 1mL of Prestozol Reagent to 1-3 × 10^7 cell nuclei, either in pellet or in suspension (volume < 0.1mL). Lyse the nuclei by inverting the assay tube or by gently pipetting the mixture.
   g) Tissue: Homogenize 25-50mg tissue in 1mL of Prestozol Reagent and incubate for 10 minutes. If required, the samples can be homogenized by using grinding pestle (Cat.No.: OWL046, not provided) before adding Prestozol Reagent.

      Note: To minimize shearing of the DNA molecules, pipette DNA solution using wide-bore pipette tips. Prepare wide bore pipette tips by cutting 2-3mm from the ends of plastic pipette tips. Mix DNA solutions by inversion; avoid shaking or use of a Vortex for mixing.
2. After incubation, centrifuge the homogenate for 10 minutes at 12,000 rpm at 4°C or room temperature. Transfer the viscous supernatant to a fresh 1.5mL microcentrifuge tube. This step removes insoluble tissue fragments, RNA, and excess polysaccharides from the lysate/homogenate. This process is recommended in order to minimize RNA carry-over into the DNA.
3. Add 0.5mL ice-cold isopropanol (95-100%) to the sample, and mix by inversion and incubate at room temperature for 2-3 minutes. DNA should quickly become visible as a cloudy precipitate. Briefly centrifuge the 1.5mL microcentrifuge tube at 12,000 rpm for 5 minutes. Carefully decant the supernatant, leaving the DNA pellet at the bottom of the tube.

4. Add 0.5mL 75% ethanol to the sample, and mix by inversion and incubate at room temperature for 2-3 minutes. Centrifuge the 1.5mL microcentrifuge tube at 12,000 rpm for 5 minutes. Decant the supernatant.
   Note: During ethanol wash, suspend the DNA in ethanol by inverting the tubes 3-6 times. Store the tubes vertically for 2-3 minutes to allow the DNA to settle to the bottom of the tubes and remove ethanol by pipetting or decanting.
5. Air-dry the DNA pellet for 30 seconds to 2 minutes after removing the ethanol in an open tube. (Exposure of DNA pellet to air for longer period will result in difficulty while dissolving) Dissolve the DNA in 8mM NaOH by slowly passing the pellet through a pipette tip. Use of the 8mM NaOH assures full solubilization of the DNA pellet. Add an adequate amount of the 8mM NaOH depending on the pellet size.
   Note: Typically add 0.2-0.5mL of 8mM NaOH to the DNA isolated 25-50mg of animal tissue. If pellet is difficult to dissolve in 8mM NaOH, then warm the sample at 50°C until the pellet is completely solubilized.
6. DNA will not be fully solubilized in TE or water. (The resolubilization of Prestozol isolated DNA is low in Tris buffers. Therefore, the use of 8mM NaOH is highly recommended.) DNA is stable in 8mM NaOH for several months at 4°C and greater than one year at -20°C.
7. Adjust the pH of DNA solution by the addition of HEPES (free acid) buffer as mentioned below.

Final pH	0.1M HEPES (µL)
8.4	86
8.2	93
8.0	101
7.8	117
7.5	159

Final pH	1M HEPES (µL)
7.2	23
7.0	32

8. The isolated DNA contains partially degraded RNA. The RNA can be digested by supplementing the mix with RNase A (10mg/mL) (Cat.No.: ORT405, not provided).

Note:
The isolation procedure can be interrupted and samples can be stored as follows: The lysate/homogenate can be stored for 18 hours at 15 to 30°C or for three days at 2 to 8°C (refer to Step 1). During washes, DNA can be stored in 75% ethanol for at least one week at 15 to 30°C or for three months at 2 to 8°C (refer to Step 4)