GenDEPOT

PureXtract RNAsol

R6101

Storage

Store at 4-20°C

Stable at 25°C for at least two years from the date of purchase.

Contents

- Product Manual
- ***PureXtract*** RNAsol

Shipping Condition

Ship at ambient.

Introduction

PureXtract RNAsol is a complete and ready -to-use reagent for the isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins from samples of human, animal, plant, yeast , bacteria, and viral origin. ***PureXtract*** RNASol combines phenol and guanidine thiovyanate in a mono-phase solution to facili -tate the imediate and most effective method of RNA isolation. It isolates a whole spectrum of RNA molecules rarely observed in RNA isolated by other methods may artificially change the mRNA composition. The entire procedure cab be completed in 1 hr and the recovery of undegraded mRNAs is 30-150% greater than with other methods of RNA isolation. ***PureXtract*** RNAsol isolates high quality RNA from diverse biological material, including animal and plant tissues rich in polysaccharides and proteoglycans.

Usage

The isolated RNA can be used for northern analysis, dot blot hybridization, poly A+ selection, in vitro translation, RNase protection assay, molecular cloning and RT-PCR. Simultaneous extraction of nearly 100% of the genomic DNA allows for normalization of the results of gene expression studies per genomic DNA instead of the more variable total RNA or tissue weight.

Precaution

PureXtract RNAsol contains a poison (phenol) and an irritant (guanidine thio -cyanate). Causes burns. CAN BE FATAL. When working with ***PureXtract*** RNAsol use gloves and eye protection (shield, safety goggles). Do not get on skin or clothing. Avoid breathing vapor.

Protocol

The procedure is carried out at room temperature, unless stated otherwise.

1. Homogenization

a. Tissues: Homogenize tissue samples in ***PureXtract*** RNAsol (1ml/50-100 mg tissue) using a glass-Teflon or polytron homogenizer. Sample volume should not exceed 10% of the volume of ***PureXtract*** RNAsol used for homogenization.

b. Cells: Cell grown in monolayer should be lysed directly in a culture dish. Pour off media, add ***PureXtract*** RNAsol and pass the cell lysate several times through a pipette. Use 1ml of ***PureXtract*** RNAsol per 10 cm^2 of culture dish area. Cells grown in suspension should be sedimented first and then lysed in ***PureXtract*** RNAsol by repetitive pipetting. Use 1.0 ml of the reagent per 5-10 $X10^6$ animal, plant or yeast cells or per 10^7 bacterial cells.

Note: Avoid washing cells before the addition of ***PureXtract*** RNAsol as this may contribute to mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. Phase seperation.

Store the monogenate for 5 minutes at room temperature to permit the com -plete dissociation of nucleoprotein complexes. Next, supplement the homo -genate with 0.1ml bromochloropropane (BCP) or 0.2ml chloroform per 1ml of ***PureXtract*** RNAsol, cover the samples tighly and shake vigorously for 15 seconds. Store the resulting mixture at room temperature for 2-15 minutes and centrifuge at room temperature for 2-15 minutes and centrifuge at 12,000g for 15 minutes at 4°C. Following centrifugation, the mixture separates into a lower red phenol -choloroform phase, interphase, and organic phase. The volume of the aqueous phase is about 60% of the volume of ***PureXtract*** RNAsol used for homogenization. Substituting BCP for chloroform does not affect the quality of isolated RNA, DNA and proteins and its use as the phase separation reagent may decrease the possibility of contaminating RNA with DNA. Chloroform used for phase separ -ation should not contain isoamyl alcohol or any other additive.

Note: It is important to perform centrifugation to separate aqueous and organic phases in the cold (4-10°C). If performed at elevated temperature, a residual amount of DNA may sequester in the aqueous phase. In this case, RNA can be used for northern analysis but it may not be suitable for PCR.

3. RNA Precipitation

Transfer the aqueous phase to a fresh tube and save the interphase and organic phase at 4°C for subsequent isolation of DNA and proteins. Precipitate RNA from the aqueous phase by mixing with isopropanol. Use 0.5ml of isopropanol per 1ml of ***PureXtract*** RNAsol used for the initial homogenization. Store samples at room temperature for 5-10 minutes and centrifuge at 12,000g for 8 minutes at 4-25°C. RNA precipitate (often invisible before centrifugation) forms a gel-like or white pellet on the side and bottom of the tube.

Note: When isolation RNA from sources rich in polysaccharises and proteoglycans, perform the modified precipitation described in the Troubleshooting Guide.

4. RNA Wash

Remove the supernatant and wash the RNA pellet (by vortexing) with 75% ethanol and subsequent centrifugation at 7,500g for 5 minutes at 4-25°C. Add at least 1ml of 75% ethanol per 1ml ***PureXtract*** RNAsol used for the initial homo -genization. **Note**: If the RNA pellet accumulates on the side of the tube and has a tendency to float, sediment the pellet at 12,000g.

5. RNA solubilization

Remove the ethanol wash and briefly air-dry the RNA pellet for 3-5 mins. It is important not to completely dry the RNA pellet as this will greatly decrease its solubility. Do not dry RNA by centrifugation under vaccum. Dissolve RNA in water or 0.5% SDS by passing the solution a few times through a pipette tip and incu -bating for 10-15 minues at 55-60°C. Water or the SDS solution used for RNA solubulization should be made RNase-free by diethyl pyrocarbonate (DEPC) treatment.

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