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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6. Results

PureXtract RNAsol sample isolates a whole spectrum of RNA moleculres rarely observed in RNA preparations isolated by other methods. Ethidium bromide staining of RNA separated in an agarose gel or methylene blue staining of a hybridization membrane after RNA transfer visualizes two predominant bands of small (~2kb) and large (~5kb) ribosomal RNA, low molecular weight (7-15kbs) RNA. The final preparation of total RNA is essentially free of DNA and proteins and has a 260-280 ratio 1.6-1.9. For RT-PCR analysis, DNase treatment may be necessary for optimal results. For optimal spectrophotometric measurements, RNA aliquots should be diluted with water buffer with a pH > 7.5 sush as Pho -sphate Buffer. Distilled water with a pH < 7.0 falsely decreases the 260-280 ratio and impedes the detection of protein contamination of RNA samples.

Expected yield:

a. Tissues (ug RNA/my tissue): liver, spleen; 6-10ug
Kidney;3-4ug
Skeletal muscles,brain; 1-5ug
Placenta; 1-4ug

b. Cultured cells (ug RNA/10^6 cells); epithelial cells; 8-15 ug
fibrolasts;5-7ug

Special protocol for isolation of DNA by *PureXtract* RNAsol

The procedure is carried out at room temperature, unless stated otherwise. The DNA is isolated from the interphase and phenol phase separated from the initial homogenate as described in the RNA isolation protocol. Following precipitation and a series of wash, the DNA is solubilized in 8mM NaOH, neutralized and used for analysis. The DNA isolated by ***PureXtract*** RNAsol can be used for PCR, restriction digestion and Southern blotting. In addition, full recovery of DNA from tissues and cultured cells permits the use of ***PureXtract*** RNAsol for determi -nation of the DNA content in analyzed samples. The procedure is carried out at room temperature, unless stated otherwise. The protocol describes isolation of DNA from the phenol phase and interphase of samples homogenized (or lysed) in 1ml of ***PureXtract*** RNAsol.

1. DNA precipitation

Remove the remaining aqueous phase overlysing the interphase. Precipitate DNA from the interphase and organic phase with ethanol. Add 0.3 ml of 100% ethanol per 1ml of ***PureXtract*** RNAsol used for the initial homogenization, and mix samples by inversion. Next store the samples at room temperature for 2-3 minutes and sediment DNA by centrifugation at 2,000g for 5 minutes at 4°C. Careful removal of any residual aqueous phase is critical for the quality of the isolated DNA.

2. DNA wash

Remove the phenol-ethanol supernatant and store at 4°C for the subsequent protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M tri -sodium citrate in 10% ethanol. (No pH adjustment required.) Use 1ml of the solution per 1ml of ***PureXtract*** RNAsol used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 minutes at room temperature with periodic mixing and centrifuge at 2,000g for 5 minutes at 4-25°C. Next, suspend the DNA pellet in 75% ethanol (1.5-2ml of 75% ethanol per 1ml ***PureXtract*** RNAsol), store for 10-20 mins at room temperature with periodic mixing and centrifuge at 2,000g for 5 minutes at 4-25°C. This ethanol wash removes pinkish color from the DNA pellet.

Note: An additional wash in 0.1M trisodium citrate -10% ethanol is required for large pellets containing >200ug DNA or large amounts of a non-DNA material.

3. DNA solubilization

Remove the ethanol wash briefly air-dry the DNA pellet by keeping tubes open for 3-5 minutes at room temperature. Dissolve the DNA pellet in 8 mM NaOH by slowly passing through a pipette. Add an adequate amount of 8mM NaOH to approach a DNA concentration of 0.2-0.3ug/ul. Typically, add 0.3-0.6ml 8mM NaOH to DNA isolated from 50-70 mg of tissue or 10^7 cells. The use of a mild alkaline solution assures full solution solubilization of the DNA pellet. At this stage, the DNA preparations (especially from tissues) still contain insoluble material (fragments of membranes, etc.); remove this material by centrifugation at 12,000g for 10 mins and transfer the resulting supernatant containing DNA to new tubes. A high viscosity of the supernatant indicates the presence of high molecular weight DNA.

*** Tips**

1. If necessary, the phenol phase and interphase can be stored at 4°C overnight. Samples suspended in 75% ethanol can be stored at 4°C for a long period of time(months). Samples solubilized in 8mM NaOH can be stored overnight at 4°C. For prolonged storage, adjust samples to pH 7-8 and supplement with 1mM EDTA.
2. Molecular weight of the isolated DNA depends on the shearing forces applied during homogenization. When possible, use a loosely fitting homogenizer. Avoid using a Polytron homogenizer.
3. The isolation protocol can be modified if the DNA is isolated only for quanti -tative purposes: a) a more vigorous homogenization of samples can be performed, including the use of Polytron: b) phenolphase and interphase can be stored at 4°C for a few days or at -70°C for a few months: c) solubilization of DNA can be facilitated by replacing 8mM NaOH with a 40mM solution and by vortexing of the DNA pellet instead of pipetting.
4. Do not shorten the recommended time of storing samples with the washing solutions. These are the minimal periods of time necessary for efficient remo -val of phenol from the DNA pellet.
5. To assure full recovery of DNA from small samples (<10ug DNA) , we reco -mmend the use of Polyacryl Carrier. Perform homogenization, phase separation and removal of the aqueous phase as described in the RNA isolation section of the protocol. Remove any remaining aqueous phase overlying the interphase and add 208ul of Polyacryl Carrier to the interphase-phenol phase. Perform DNA precipitation as described in Step 1 of the DNA isolation procedure. Replace the sodium citrate washes described in Step 2 by performing two 10 minutes washes of the DNA/carrier pellet using 75% ethanol with intermittent mixing. Proceed with DNA solubilization as described in the protocol.

This alternative procedure replaces steps 1-2 of the DNA isolation procedure. Prepare a back extraction buffer containing; 4M guanidine thiocyanate, 50mM sodium citrate and 1M Tris (free base). Following phase separation (RNA iso -lation preocedure Step 2), remove any remaning aqueous phase overlying the interphase and add back extraction buffer to the interphase-organic phase mixture. Use 0.5ml of back extraction buffer per 1.9ml of ***PureXtract*** RNAsol used for the initial homogenization. Vigorously mix the sample by inversion

for 15 secs and store for 10 mins at room temperature. Perform phase seperation by centrifugation at 12,000g for 15 mins at 4°C. Transfer the upper aqueous phase containing DNA to a clean tube and save the interphase and organic phase at 4°C for subsequent protein isolation. Precipitate DNA from the aqueous phase by adding 0.4ml of isopropanol per 1.9ml of ***PureXtract*** RNAsol used for the initial homogenization. Mix the sample by inversion and store for 5 mins at room temperature. If the expected DNA yield is less than 20ug, add 2-8ug of Polycryl Carrier to the aqueous phase prior to isopropanol addition and mix. Sediment DNA by centrifugation at 12,000g for 5 mins at 4-25°C and remove the supernatant. Wash the DNA pellet with 1.0ml of 75% ethanol and proceed with DNA solubilization as described in Step 3.

Special protocol for isolation of Protein by *PureXtract* RNAsol

Proteins are isolated from the phenol-ethanol supernatant obtained after preci -pitation of DNA with ethanol (step 1, DNA precipitation). The resulting prepara -tion can be analyzed for the presence of specific proteins by Western blotting. The procedure is carried out at room temperature unless stated otherwise.

1. Protein precipitation

Aliquot a portion of the phenol-ethanol supernatant (0.2-0.5ml, 1 volume) into a microfuge tube. Precipitate proteins by adding 3 volumes of acetone. Mix by inversion for 10-15 sec to obtain a homogeneous solution. Store samples for 10 mins at room temperature and sediment the protein precipitate at 12,000g for 10 mins at 4°C (See tips 1 and 2).

2. Protein wash

Decant the phenol-ethanol supernatant and disperse the protein pellet in 0.5ml of 0.3M guanidine hydrochloride in 95% ethanol + 2.5% glycerol (V:V). Disperse the pellet using a pipette tip or syringe needle attached to a mechanical stirrer (~30 sec at 800-1,000 RPM). After dispersing the pellet, add another 0.5ml aliquot of the guanidine hydrochloride/ethanol/glycerol wash solution and perform two more washes in 1ml each of the guanidine/ethanol/glycerol wash solution. Disperse the pellet by vortexing after each wash to efficiently remove residual phenol. Perform the final wash in 1ml of ethanol containing 2.5% glycerol (V:V). At the end of the 10 mins ethanol wash, sediment the protein at 8,000g for 5 mins. Decant the alcohol, invert the tube and dry the pellet for 7-10 mins at room tempera -ture (See Tips 3).

3. Protein solubilization

Option 1. After briefly air-drying the protein pellet, add a suitable solvent such as 1% SDS, 10M urea, or another suitable detergent-based solvent to the protein pellet. Use 0.2ml of solvent per 10-20mg of tissue sample (See tips 4). Gently disperse and solubilize the pellet for 15-20 minutes by “flicking” the tube or pipetting as required. The addition of a suitable reducing agent such as tributyl -phosphine (2.5% solution volume) will improve protein yield in most preparations. For immediate use in western analysis, heat the solution for 3 min at 100°C and sediment any insoluble material by centrifugation at 10,000g for 5 mins at RT. Transfer the supernatant to a clean tube and use the protein immediately for western blotting (See tips 5). Otherwise, store the solubilized proteins at -20°C and perform the heating, centrifugation or other preparatory steps at the time of use.

Option 2. Dialyze the phenol-ethanol supernatant (II DNA isolation Step 1, DNA Precipitate) in a suitable, regenerated cellulose dialysis tubing against three changes of 0.1% SDS at 4°C. Centrifuge the dialysate at 10,000g for 10 mins at 4°C and use the clear supernatant for Western blotting.

*** Tips**

1. Isopropanol may replace acetone during protein precipitation but total re -covered protein yield may be reduced by 5-10%.
2. Limiting the volume of phenol-ethanol supernatant to 0.2-0.5ml per tube will produce a smaller, more manageable protein pellet and improve protein yield. ***PureXtract*** RNAsol protein extracts prepared from rat tissues yield 50 -110ug protein/mg tissue.
3. In general, protein pellets suspended in 0.3M guanidine hyrochloride/ethanol /glycerol wash solution or in ethanol glycerol wash solution can be stored for at least one month at 4°C or one year -20°C. Individual proteins may display different sensitivity to long-term storage and optimal storage conditions should be established for sensitive and labile proteins.
4. The solubility and stability of specific proteins can be influenced by diffferent detergent solutions. To obtain optimal results in various experimental applica -tions, investigators may solubilize which solution best addressed their unique experimental objectives.
5. Solubilize protein may form insoluble aggregates during storage at -20°C. Prior to western analysis, thaw the samples at 25°C for 10-15 minutes. Heat the solu -bilized protein sample for 3 min at 100°C, pipette the solution and remove insoluble protein by centrifugation as outlined in the protocol.

Troubleshooting guide

RNA isolation

1. Low yield

a) incomplete homogenization of lysis of samples.
b) incomplete solubilization of the final RNA pellet.

2. 260/280 ratio <1.6

a. too small volume of the reagent used for sample homogenization.
b) acidic water was used for the spectrophotometric measurement.
c) contamination of the aqueous phase with phenol phase.
d) incomplete solubilization of the final RNA pellet.

3. RNA degradation

a) tissues were not immediately processed or frozen after removing from animal
b) samples used for isolation or the isolated DNA preparations were stored at -20°C instead of at -70°C
c) cells were dispersed by trypsin digestions
d) aqueous solutions or tubes used for solubilization of RNA were not RNase-free
e) formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

4. DNA contamination

a) too small volume of the reagent was used for sample homogenization.
b) samples used for the isolation contained organic solvents. (ethanol, DMSO).
c) phase seperation was performed at temperature above 10°C.

5. Proteoglycan and polysaccharide contamination.

The following modification of RNA precipitation (Step 3) removes these contami -nating compounds from the isolated RNA. Add to the aqueous phase 0.25ml of isopropanol followed by 0.25ml of a High Salt Precipitation Solution (0.8M sodium citrate and 1.2M NaCl) per 1ml of ***PureXtract*** RNASol used for the homogenization Mix the solution, store it for 5-10 minutes at room temperature and centrifuge at

12,000g for 8 mins at 4-25°C. Wash the resulting RNA pellet as described in Step 4 of the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form. To isolate pure of polysaccharides, the modified precipitation should be combined with an additional centrifugation of the initial homogenate described in Tip 5 in the RNA isolation protocol.

DNA isolation

1. Low Yield

a) incomplete homogenization or lysis of samples.

b) incomplete solubilization of the final DNA pellet.

2. 260/280 ratio<1.70

a) phenol was not sufficiently removed from the DNA preparation.

b) acidic water was used for the spectrophotometric measurement.

3. DNA degradation

a) tissues were not immediately processed or frozen after removing from animal

b) samples were homogenized with a Polytron or other high speed homogenizer.

4. RNA contamination

a) too large volume of aqueous phase remained with the interphase and organic phase

b) DNA pellet was not sufficiently washed with 10% ethanol - 0.1M sodium citrate solution.

Protein Isolation

1. Low yield

a) incomplete homogenization of lysis of samples.

b) incomplete solubilization of the final protein pellet.

2. Protein degradation

Tissues were not immediately processed or frozen after removing from animal

3. Band deformation in PAGE

Insufficient wash of the protein pellet.

Related products

Description	Cat No
amfiRivert Reverse Transcriptase	A1202
dNTP mixture, 10mM each	D0610
Oligo(dT)$_{18}$	O1024
RNase Inhibitor Plus	R2808
Random Hexamer	R3100
amfiRivert cDNA Synthesis Platinum Master Mix	R5600
gRazor, Genomic DNA Removal Enzyme Mix(5X)	R5800
PureXtract RNAsol, Trizol equivalent	R6101
RNazor, RNase Decontamination Reagent, Spray bottle	R7000
DEPC Treated Water	W0805