PRODUCT: **TRI REAGENT® LS - RNA / DNA / PROTEIN ISOLATION REAGENT for LIQUID SAMPLES**
Cat. No: TS 120 March 2017
Storage: Store at 4 - 25 C

PRODUCT DESCRIPTION

TRI Reagent® LS is a complete and ready to use reagent for the isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins from liquid samples of human, animal, plant, yeast, bacterial and viral origin. TRI Reagent® LS is the improved version of the popular single-step method of total RNA isolation (1, 2). This highly reliable technique performs well with small and large sample volumes, and allows simultaneous processing of a large number of samples. TRI Reagent® LS and the single-step method are subjects of the international patents.

TRI Reagent® LS combines phenol and guanidine thiocyanate in a mono-phase solution to facilitate the immediate and most effective inhibition of RNase activity. A biological sample is homogenized or lysed in TRI Reagent® LS and the lysate is separated into aqueous and organic phases by bromochloropropane or chloroform addition and centrifugation. RNA remains exclusively in the aqueous phase, DNA in the interphase, and proteins in the organic phase. RNA is precipitated from the aqueous phase by the addition of isopropanol, and the RNA pellet is washed with ethanol and solubilized. DNA and proteins are sequentially precipitated from the interphase and organic phase with ethanol and isopropanol, washed with ethanol and solubilized.

STABILITY: TRI REAGENT® LS is stable at 25 C for at least two years from the date of purchase (3).

SPECIAL HANDLING PRECAUTIONS

TRI Reagent® LS contains a poison (phenol) and an irritant (guanidine thiocyanate). Causes burns. CAN BE FATAL. When working with TRI Reagent® LS, **use gloves and eye protection** (shield, safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read the warning note on the bottle and SDS. **In case of contact:** Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek immediate medical attention.

I. ISOLATION OF RNA

TRI Reagent® LS is the most effective method of RNA isolation. **The entire procedure can be completed in 1 hour and the recovery of undegraded mRNAs is 30 - 150% greater than with other methods of RNA isolation**. TRI Reagent® LS isolates a whole spectrum of RNA molecules rarely observed in RNA isolated by other methods. Typically, column-based methods may artificially change the mRNA composition. TRI Reagent® LS isolates RNA from diverse biological material including animal and plant tissues rich in polysaccharides and proteoglycans. The isolated RNA and 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 to genomic DNA instead of the more variable total RNA or tissue weight.

PROTOCOL

Reagents required, but not supplied: chloroform or 1-bromo-3-chloropropane (BCP, cat. no. BP 151), isopropanol and ethanol. We recommend the use of disposable polypropylene tubes provided by Molecular Research Center, Inc. Tubes from other suppliers should be tested to ensure integrity during centrifugation at 12,000 g with TRI Reagent® LS.
The protocol includes the following steps:

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1. HOMOGENIZATION - 0.75 ml TRI Reagent LS + 0.25 ml sample.
2. PHASE SEPARATION - homogenate + 0.1 ml BCP or 0.2 ml chloroform.
3. RNA PRECIPITATION - aqueous phase + 0.5 ml isopropanol.
4. RNA WASH - 1 ml 75% ethanol.
5. RNA SOLUBILIZATION - FORMAzol®, 0.5% SDS, or water.

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

1. HOMOGENIZATION

A. BIOLOGICAL FLUIDS. Mix 0.75 ml of TRI Reagent LS with 0.25 ml of sample and lyse cells (or cellular debris) suspended in the sample by passing the suspension several times through a pipette. Use at least 0.75 ml of TRI Reagent LS per 5 - 10 x 10^6 cells.
B. TISSUE SUSPENSIONS. Homogenize 0.25 ml sample with 0.75 ml of TRI Reagent LS in a glass-Teflon or Polytron homogenizer.
If the sample volume is < 0.25 ml, adjust the volume to 0.25 ml with water. The volume ratio of TRI Reagent LS to sample should be 3 to 1. For the isolation of RNA from cells grown in monolayer see Note 3 in the Notes For RNA Isolation.

2. PHASE SEPARATION

Store the lysate/homogenate for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. Supplement the lysate with 0.1 ml BCP or 0.2 ml chloroform per 0.75 ml of TRI Reagent LS. Cover the sample tightly and shake vigorously for 15 seconds. Store the resulting mixture at room temperature for 2 - 15 minutes. Centrifuge the sample at 12,000 g for 15 minutes at 4 C. Following centrifugation, the mixture separates into a lower red phenol phase, interphase, and the upper colorless aqueous phase. RNA remains exclusively in the aqueous phase whereas DNA and proteins are in the interphase and organic phase. The volume of the aqueous phase is about 70% of the volume of TRI Reagent LS used for homogenization.

BCP is less toxic than chloroform and its use reduces the possibility of contaminating RNA with DNA (4). Chloroform used for phase separation should not contain isoamyl alcohol or any other additive.

It is important to perform centrifugation for phase separation in the cold (4 - 10 C). If performed at elevated temperatures, 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 0.5 ml of isopropanol per 0.75 ml of TRI Reagent LS used for the initial homogenization. Store samples at room temperature for 5 - 10 minutes and centrifuge at 12,000 g 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.

When isolating RNA from sources rich in polysaccharides and proteoglycans, perform the modified precipitation described in the Troubleshooting guide (last page).

4. RNA WASH

Remove the supernatant and wash the RNA pellet with 75% ethanol by vortexing and subsequent centrifugation at 7,500 g for 5 minutes at 4 - 25 C. Add at least 1 ml of 75% ethanol per 0.75 ml of TRI Reagent LS used for the initial homogenization. If the RNA pellet accumulates on the side of the tube and has a tendency to float, perform the ethanol wash at 12,000 g.

5. RNA SOLUBILIZATION

Remove the ethanol wash and briefly air-dry the RNA pellet for 3 - 5 minutes. It is important to avoid completely drying the RNA pellet as this will greatly decrease its solubility. **Do not dry RNA by centrifugation under vacuum**. Drying is not necessary for solubilization of RNA in FORMAzol® (stabilized formamide, cat. no. FO-121). Dissolve RNA in FORMAzol, water or 0.5% SDS by passing the solution a few times through a pipette tip and incubating for 10 - 15 minutes at 55 - 60 C. Water or the SDS solution used for RNA solubilization should be made RNase-free by diethyl pyrocarbonate (DEPC) treatment. RNA should be precipitated from FORMAzol with ethanol before using for RT-PCR.

6. RESULTS

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 (~2 kb) and large (~5 kb) ribosomal RNA, low molecular weight (0.1-0.3 kb) RNA, and discrete bands of high molecular weight (7 - 15 kb) 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 or buffer with a pH > 7.5. Distilled water with a pH < 7.0 falsely decreases the 260/280 ratio and impedes the detection of protein contamination in RNA samples (7).

NOTES FOR RNA ISOLATION

1. To facilitate isolation of small amounts of RNA (<10 µg) perform homogenization (or lysis) in 0.75 ml of TRI Reagent® LS supplemented with 1 - 4 µl of Polyacryl Carrier™ (cat. no. PC 152). If the sample volume is < 0.25 ml, adjust the volume to 0.25 ml with water. Following homogenization, add BCP or chloroform and proceed with the phase separation and other steps of isolation as described above.
2. After homogenization (before addition of BCP) samples can be stored at -70 C for at least two years. The RNA precipitate (Step 4, RNA Wash) can be stored in 75% ethanol at 4 C for at least one week, or at least two years at -20 C.
3. When isolating RNA from cells grown in monolayer, use 0.3 - 0.4 ml of TRI Reagent® LS per 10 cm^2 area of a culture dish. In this application, do not supplement TRI Reagent® LS with water. The leftover medium adhering to a culture dish adequately dilutes the reagent. Lyse cells by repetitive pipetting and perform phase separation as described above.
4. Hands and dust may be a major source of the RNase contamination. Use gloves and keep tubes closed throughout the procedure.
5. An additional isolation step may be required for samples with a high content of proteins, fat, polysaccharides or extracellular material. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 g for 10 minutes at 4 C. The resulting pellet contains extracellular membranes, polysaccharides and high molecular weight DNA while the supernatant contains RNA. In samples from fat tissue, an excess of lipid collects as a top layer which should be removed. Transfer the clear supernatant to a fresh tube and proceed with the phase separation and other steps of RNA isolation as described above. High molecular weight DNA can be recovered from the pellet by following Steps 2 and 3 of the DNA Isolation Protocol.
6. See also Troubleshooting Guide, poly A^+ RNA isolation and RT-PCR application notes on the last page of this booklet.

II. ISOLATION OF DNA BY TRI REAGENT® LS

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 washes, the DNA is solubilized in 8 mM NaOH, neutralized and used for analysis. The DNA isolated by TRI Reagent® LS can be used for PCR, restriction digestion and Southern blotting. In addition, full recovery of DNA from tissues and cell suspensions permits the use of TRI Reagent® LS for determination of the DNA content in analyzed samples (2).

PROTOCOL

Reagents required, but not supplied: ethanol, sodium citrate and sodium hydroxide.
The protocol includes the following steps:

1. DNA PRECIPITATION	- phenol phase and interphase + 0.3 ml ethanol (0.75 ml TRI Reagent LS).
2. DNA WASH	- 1 ml 0.1 M sodium citrate in 10% ethanol x 2.
	- 2 ml 75% ethanol.
3. DNA SOLUBILIZATION	- 8 mM NaOH.

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 1 ml of TRI Reagent® LS.

1. DNA PRECIPITATION

Remove the remaining aqueous phase overlying the interphase. Precipitate DNA from the interphase and organic phase with ethanol. Add 0.3 ml of 100% ethanol per 0.75 ml of TRI Reagent LS used for the initial homogenization, and mix samples by inversion. Store the samples at room temperature for 2 - 3 minutes and sediment DNA by centrifugation at 2,000 g for 5 minutes at 4 C. Careful removal of any residual aqueous phase is critical for the quality of the isolated DNA.
See Notes 6 for an alternative DNA isolation procedure.

2. DNA WASH

Remove the phenol-ethanol supernatant and save it at 4 C for the protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M sodium citrate in 10% ethanol (no pH adjustment required). Use 1 ml of the solution per 0.75 ml of TRI Reagent LS 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 2,000 g for 5 minutes at 4 - 25 C. Following the two washes, suspend the DNA pellet in 75% ethanol (1.5 - 2 ml of 75% ethanol per 0.75 ml TRI Reagent LS), store for 10 - 20 minutes at room temperature with periodic mixing and centrifuge at 2,000 g for 5 minutes at 4 - 25 C. This ethanol wash the pinkish color from the DNA pellet.
An additional wash in 0.1M sodium citrate-10% ethanol is required for large pellets containing >200 µg DNA or large amounts of a non-DNA material.

3. DNA SOLUBILIZATION

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

QUANTITATION OF DNA

For optimal spectrophotometric measurements, DNA aliquots should be diluted with water or buffer with a pH >7.5. Distilled water with a pH < 7.0 falsely decreases the 260/280 ratio and impedes the detection of protein in RNA samples (7).

Calculate the DNA content assuming that one A_{260} unit equals 50 µg of double-stranded DNA/ml. For calculation of the cell number, assume that the amount of DNA per 10^6 of diploid cells of human, rat and mouse origin equals 7.1 µg, 6.5 µg and 5.8 µg, respectively (5). A typical preparation of DNA isolated from tissues is composed of 60 - 100 kb DNA (70%) and ~20 kb DNA (30%). Preparations isolated from cultured cells contain >80% of 60 - 100 kb DNA and < 20% of 20 kb DNA. The isolated DNA is free of RNA and proteins and has a 260/280 ratio >1.7.

AMPLIFICATION OF DNA BY PCR

Following solubilization in 8 mM NaOH, adjust the pH of the DNA sample to 8.4 using HEPES (see Table). Add an aliquot of the sample (typically 0.1 - 1 µg DNA) to a PCR reaction mix and perform PCR according to your standard protocol.

DIGESTION OF DNA BY RESTRICTASES

Adjust the pH of the DNA solution to a required value using HEPES (see Table). Alternatively, dialyze samples against 1 mM EDTA, pH 7 - 8. Carry out the DNA restriction for 3 - 24 hours under optimal conditions for a specific restrictase using 3 - 5 units of the enzyme per µg DNA. In a typical assay, 80 - 100% of the DNA preparation is digested by restrictases.

Adjustment of pH in DNA samples solubilized in 8 mM NaOH.

For 1 ml of 8 mM NaOH use the following amounts of 0.1 M or 1 M HEPES (free acid):

Final pH	0.1 M HEPES (µl)	Final pH	0.1 M HEPES (µl)	Final pH	1 M HEPES (µl)
8.4	86	7.8	117	7.2	23
8.2	93	7.5	159	7.0	32
8.0	101				

NOTES FOR DNA ISOLATION

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 8 mM NaOH can be stored overnight at 4 C. For prolonged storage, adjust samples to pH 7 - 8 and supplement with 1 mM 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 quantitative purposes: a) a more vigorous homogenization of samples can be performed, including the use of Polytron; b) phenol phase 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 8 mM NaOH with a 40 mM 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 removal of phenol from the DNA pellet.
5. To assure full recovery of DNA from small samples (< 10 µg DNA), we recommend the use of Polyacryl Carrier™ (cat. no. PC 152). Perform homogenization, phase separation and removal of the aqueous phase as described in the RNA Isolation protocol. Remove any remaining aqueous phase overlying the interphase and add 1 - 4 µl 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 minute washes of the DNA/carrier pellet using 75% ethanol with intermittent mixing. Proceed with DNA solubilization as described in the protocol.
6. This alternative procedure replaces Steps 1 - 2 of the DNA Isolation procedure. Prepare a back extraction buffer containing: 4 M guanidine thiocyanate, 50 mM sodium citrate and 1 M Tris (free base). Following phase separation (RNA Isolation Procedure Step 2), remove any remaining aqueous phase overlying the interphase and add back extraction buffer to the interphase - organic phase mixture. Use 0.5 ml of back extraction buffer per 0.75 ml of TRI Reagent LS used for the initial homogenization. Vigorously mix the sample by inversion for 15 seconds and store for 10 minutes at room temperature. Perform phase separation by centrifugation at 12,000 g for 15 minutes 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 0.4 ml of isopropanol per 0.75 ml of TRI Reagent LS used for the initial homogenization. Mix the sample by inversion and store for 5 minutes at room temperature. If the expected DNA yield is less than 20 µg, add 1 - 4 µl of Polyacryl Carrier to the aqueous phase prior to isopropanol addition and mix. Sediment DNA by centrifugation at 12,000 g for 5 minutes at 4 - 25 C and remove the supernatant. Wash the DNA pellet with 1.0 ml of 75% ethanol and proceed with DNA solubilization as described in Step 3.
7. Also see the Troubleshooting Guide on the last page of this booklet.

III. ISOLATION OF PROTEINS BY TRI REAGENT® LS

Proteins are isolated from the phenol-ethanol supernatant obtained after precipitation of DNA with ethanol (DNA Isolation Procedure, Step 1). The resulting preparation can be analyzed for the presence of specific proteins by Western blotting (2, 8, 9).

PROTOCOL

Reagents required but not supplied: guanidine hydrochloride, ethanol, isopropanol, acetone, glycerol, SDS, urea and tributylphosphine (Sigma T 7567).
The protocol includes the following steps:

1. PROTEIN PRECIPITATION	- 0.2 - 0.5 ml phenol-ethanol supernatant (1 volume) + acetone (3 volumes)
2. PROTEIN WASH	- 1 ml of guanidine hydrochloride/ethanol/glycerol wash solution, 3 x 10 min.; - 1 ml ethanol/glycerol solution, 1 x 10 min.
3. PROTEIN SOLUBILIZATION	- 1% SDS, 10M Urea or other suitable solvent

The procedure is carried out at room temperature unless stated otherwise. This protocol describes the isolation of proteins from the phenol-ethanol supernatant obtained from a sample homogenized in TRI Reagent® LS.

1. PROTEIN PRECIPITATION

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

2. PROTEIN WASH

Decant the phenol-ethanol supernatant and disperse the protein pellet in 0.5 ml of 0.3 M guanidine hydrochloride in 95% ethanol + 2.5 % glycerol (V:V). Disperse the pellet using a pipette tip, syringe needle or a small conical Teflon pestle (Fisher K749515-0000) attached to a mechanical stirrer (~30 sec @ 800-1000 RPM). After dispersing the pellet, add another 0.5 ml aliquot of the guanidine hydrochloride/ethanol/glycerol wash solution and store for 10 minutes at room temperature. Sediment the protein at 8,000 g for 5 minutes. Decant the wash solution and perform two more washes in 1 ml 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 1 ml of ethanol containing 2.5 % glycerol (V:V). At the end of the 10 minute ethanol wash, sediment the protein at 8,000 g for 5 minutes at 4 C. Decant the alcohol, invert the tube and dry the pellet for 7 - 10 minutes at room temperature (See Note 3).

3. PROTEIN SOLUBILIZATION

Option 1. After briefly air-drying the protein pellet, add a suitable solvent such as 1% SDS, 10 M urea, or another suitable detergent-based solvent to the protein pellet (9). Use 0.2 ml of solvent per 10 - 20 mg of tissue sample (See Note 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 tributylphosphine (2.5% of solution volume) will improve protein yield in most preparations. For immediate use in Western analysis, heat the solution for 3 minutes at 100 C and sediment any insoluble material by centrifugation at 10,000 g for 5 minutes at room temperature. Transfer the supernatant to a clean tube and use immediately for Western blotting (See Note 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 (DNA Isolation Protocol Step 1) in a suitable, regenerated cellulose dialysis tubing against three changes of 0.1% SDS at 4 C. Centrifuge the dialysate at 10,000 g for 10 minutes at 4 C and use the clear supernatant for Western blotting.

NOTES FOR PROTEIN ISOLATION

1. Isopropanol may replace acetone during protein precipitation but total recovered protein yield may be reduced by 5 - 10 % (8).
2. Limiting the volume of phenol - ethanol supernatant to 0.2 - 0.5 ml per tube will produce a smaller, more manageable protein pellet and improve protein yield.
3. In general, protein pellets suspended in 0.3 M guanidine hydrochloride/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 different detergent solutions (9). To obtain optimal results in various experimental applications, investigators may solubilize small amounts of protein in different solvents and determine which solution best addresses their unique experimental objectives.
5. Solubilized 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 solubilized protein sample for 3 minutes at 100 C, pipette the solution and remove insoluble protein by centrifugation as outlined in the protocol.

IV. TROUBLESHOOTING GUIDE.

RNA ISOLATION

Low yield. a) incomplete homogenization or lysis of samples, b) incomplete solubilization of the final RNA pellet.
260/280 ratio < 1.6. a) too small a 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.
RNA degradation. a) tissues were not immediately processed or frozen after removing from animal, b) samples used for isolation, or the isolated RNA preparations were stored at -20 C instead of at -70 C, c) cells were dispersed by trypsin digestion, 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.
DNA contamination. a) too small a volume of the reagent was used for homogenization, b) samples used for the isolation contained organic solvents, strong buffers or alkaline solution, c) phase separation was performed at temperatures above 10 C.
Proteoglycan and polysaccharide contamination. The following modification of RNA precipitation (Step 3) removes these contaminating compounds from the isolated RNA (6). Add to the aqueous phase 0.25 ml of isopropanol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 0.75 ml of TRI Reagent® LS used for the homogenization. Mix the solution, store it for 5 - 10 minutes at room temperature, and centrifuge at 12,000 g for 8 minutes 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 RNA from plant material containing a very high level of polysaccharides, the modified precipitation should be combined with an additional centrifugation of the initial homogenate described in Note 5 for the RNA Isolation Protocol.

DNA ISOLATION

Low yield. a) incomplete homogenization or lysis of samples, b) incomplete solubilization of the final DNA pellet.
260/280 ratio < 1.70. a) phenol was not sufficiently removed from the DNA preparation, b) acidic water was used for the spectrophotometric measurement, c) incomplete solubilization of the DNA pellet.
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.
RNA contamination. a) too large a volume of aqueous phase remained with the interphase and organic phase, b) DNA pellet was not sufficiently washed with 10% ethanol - 0.1 M sodium citrate solution.

PROTEIN ISOLATION

Low yield. a) incomplete homogenization or lysis of samples, b) incomplete solubilization of the final protein pellet.
Protein degradation. Tissues were not immediately processed or frozen after removing from animal.
Band deformation in PAGE. Insufficient wash of the protein pellet.

V. ISOLATION OF POLY A^+ RNA

Following RNA precipitation with isopropanol (RNA Isolation Protocol Step 3), the RNA pellet can be dissolved in a poly A^+ binding buffer and poly A^+ RNA selection can be performed on an oligo-dT column, or using any commercial product according to a standard protocol of Aviv and Leder (Proc Natl Acad Sci USA, 1972, 69, 1408-1412).

VI. RT-PCR APPLICATION NOTES

The additional centrifugation described in Note 5 for the RNA Isolation Protocol further eliminates the possibility of DNA contamination of RNA extracted by TRI Reagent® LS. A more complete evaporation of ethanol is required for RNA samples used in RT-PCR. This is especially important for small volume samples (5 - 20 µl) which, if not dried sufficiently, may contain a relatively high level of ethanol.

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