PRODUCT: **TRI REAGENT® - RNA / DNA / PROTEIN ISOLATION REAGENT**
Cat. No: TR 118
Storage: Store at 4 - 25 C

PRODUCT DESCRIPTION

TRI REAGENT® 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, bacterial and viral origin. TRI REAGENT 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 quantities of tissues or cultured cells, and allows simultaneous processing of a large number of samples. TRI REAGENT and the single-step method are subjects of the international patents.

TRI REAGENT 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 and the homogenate 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 addition of isopropanol, 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 is stable at 25 C for at least two years from the date of purchase (3).

SPECIAL HANDLING PRECAUTIONS

TRI REAGENT contains a poison (phenol) and an irritant (guanidine thiocyanate). Causes burns. CAN BE FATAL. When working with TRI REAGENT **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 MSDS. **In case of contact:** Immediately flush eyes or skin with a large amount of water for at least 15 min and seek immediate medical attention.

I. ISOLATION OF RNA

TRI REAGENT is the most effective method of RNA isolation. It isolates a whole spectrum of RNA molecules rarely observed in RNA isolated by other methods. Typically, the column-based methods may artificially change the mRNA composition. **The TRI REAGENT procedure can be completed in 1 hr and the recovery of undegraded mRNAs is 30-150% greater than with other methods of RNA isolation**. TRI REAGENT isolates high quality RNA from diverse biological material, including animal and plant tissues rich in polysaccharides and proteoglycans. 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.

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.
The protocol includes the following steps:

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1. HOMOGENIZATION - 1 ml TRI REAGENT + 50 - 100 mg tissue, 5 - 10 x 10^6 cells or 10 cm^2 of culture plate.
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. TISSUES. Homogenize tissue samples in TRI REAGENT (1 ml/50 - 100 mg tissue) using a glass-Teflon or Polytron homogenizer. **Sample volume should not exceed 10% of the volume of TRI REAGENT** used for homogenization.

B. CELLS. Cells grown in monolayer should be lysed directly in a culture dish. Pour off media, add TRI REAGENT and pass the cell lysate several times through a pipette. Use 1 ml of TRI REAGENT per 10 cm^2 of culture dish area. See also Note 3 in Notes to the RNA isolation protocol.
Cells grown in suspension should be sedimented first, and then lysed in TRI REAGENT by repetitive pipetting. Use 1.0 ml of the reagent per 5 - 10 x 10^6 animal, plant or yeast cells or per 10^7 bacterial cells.
Avoid washing cells before the addition of TRI REAGENT as this may contribute to mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. PHASE SEPARATION

Store the homogenate for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. Supplement the homogenate with 0.1 ml BCP or 0.2 ml chloroform per 1 ml of TRI REAGENT, cover the samples tightly and shake vigorously for 15 seconds. Store the resulting mixture at room temperature for 2 - 15 minutes and centrifuge at 12,000 g for 15 minutes at 4 C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, interphase, and the colorless upper 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 60% of the volume of TRI REAGENT used for homogenization.

Substituting BCP for chloroform does not affect the quality of isolated RNA, DNA or proteins and its use as a phase separation reagent may decrease 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 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 0.5 ml of isopropanol per 1 ml of TRI REAGENT 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 RNA pellet (by vortexing) with 75% ethanol and subsequent centrifugation at 7,500 g for 5 minutes at 4 - 25 C. Add at least 1 ml of 75% ethanol per 1 ml TRI REAGENT used for the initial homogenization. If the RNA pellet accumulates on the side of the tube or has a tendency to float, sediment the pellet at 12,000 g.

5. RNA SOLUBILIZATION

Remove the ethanol wash and briefly air-dry the RNA pellet for 3 - 5 min. It is important not to completely dry 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).

Expected Yield: A) tissues (µg RNA/mg tissue): liver, spleen, 6 - 10 µg; kidney, 3 - 4 µg; skeletal muscles, brain, 1 - 1.5 µg; placenta, 1 - 4 µg; B) cultured cells (µg RNA/10^6 cells): epithelial cells, 8 - 15 µg; fibroblasts, 5 - 7 µg.

NOTES

1. To facilitate isolation of RNA from small samples (<10^6 cells or <10 mg tissue) perform homogenization (or lysis) in 0.8 ml of TRI REAGENT supplemented with 2 - 8 µl of Polyacryl Carrier™ (cat. no. PC 152). Next, add BCP or chloroform and proceed with the phase separation and other steps of isolation as described above.
2. After homogenization (before addition of chloroform) samples can be stored at -70 C for at least one month. The RNA precipitate (step 4, RNA WASH) can be stored in 75% ethanol at 4 C for at least one week, or at least one year at -20 C.
3. **For cells grown in monolayer, use the amount of TRI REAGENT based on the area of a culture dish and not on cell number.** The use of an insufficient amount of TRI REAGENT may result in contamination of the isolated RNA with DNA.
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 such as muscles, fat tissue and tuberous parts of plants. 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 fat 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.
7. Liquid samples (amniotic fluid, serum, whole blood) should be processed using TRI REAGENT LS (TS 120) or TRI REAGENT BD (TB 126).

II. ISOLATION OF DNA BY TRI REAGENT

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 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 TRI REAGENT for determination of the DNA content in analyzed samples (2).

PROTOCOL

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

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1. DNA PRECIPITATION - phenol phase and interphase + 0.3 ml ethanol (1 ml TRI REAGENT).
2. DNA WASH - 1 ml 0.1 M trisodium 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.

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 1 ml of TRI REAGENT 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 2,000 g for 5 minutes at 4 C. Careful removal of any residual aqueous phase is critical for the quality of the isolated DNA.
See Note 6 for an alternative DNA isolation procedure.

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 trisodium citrate in 10% ethanol (no pH adjustment required). Use 1 ml of the solution per 1 ml of TRI REAGENT 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. Next, suspend the DNA pellet in 75% ethanol (1.5 - 2 ml of 75% ethanol per 1 ml TRI REAGENT), store for 10 - 20 min at room temperature with periodic mixing and centrifuge at 2,000 g for 5 minutes at 4 - 25 C. This ethanol wash removes pinkish color from the DNA pellet.
An additional wash in 0.1M trisodium 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 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 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 50 - 70 mg of tissue or 10^7 cells. The use of a mild alkaline solution assures full 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 12,000 g for 10 minutes and transfer the resulting supernatant containing DNA to new tubes. 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 in analyzed samples, 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% ~ 20 kb DNA. The isolated DNA is free of RNA and proteins and has a 260/280 ratio > 1.7.

Expected yield: A) Tissues (μg DNA/mg tissue): liver, kidney, 3 - 4 μg: skeletal muscles, brain, placenta 2 - 3 μg. B) Cultured human, rat and mouse cells 5 - 7 ug DNA/10^6 cells).

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 - pH 8. Carry out the DNA restriction for 3 - 24 h 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

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 section of the protocol. Remove any remaining aqueous phase overlying the interphase and add 2 - 8 ul 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 1.0 ml of TRI Reagent used for the initial homogenization. Vigorously mix the sample by inversion for 15 sec and store for 10 min at room temperature. Perform phase separation by centrifugation at 12,000 g for 15 min 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 1.0 ml of TRI Reagent used for the initial homogenization. Mix the sample by inversion and store for 5 min at room temperature. If the expected DNA yield is less than 20 µg, add 2 - 8 µl of Polyacryl Carrier to the aqueous phase prior to isopropanol addition and mix. Sediment DNA by centrifugation at 12,000 g for 5 min 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

Proteins are isolated from the phenol-ethanol supernatant obtained after precipitation of DNA with ethanol (Step 1, DNA PRECIPITATION). 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.

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 sec to obtain a homogeneous solution. Store samples for 10 min at room temperature and sediment the protein precipitate at 12,000 g for 10 min 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 pipet 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 to the sample and store for 10 min at RT. Sediment the protein at 8,000 g for 5 min. 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 min ethanol wash, sediment the protein at 8,000 g for 5 min at 4 C. Decant the alcohol, invert the tube and dry the pellet for 7-10 min 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 min at 100 C and sediment any insoluble material by centrifugation at 10,000 g for 5 min at RT. Transfer the supernatant to a clean tube and use the protein solution 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 (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 10,000 g for 10 min at 4 C and use the clear supernatant for Western blotting.

NOTES

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. TRI Reagent protein extracts prepared from rat tissues yield 50 – 110 µg protein / mg tissue.
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 min. Heat the solubilized protein sample for 3 min 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 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 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 (ref.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 1 ml of TRI Reagent 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 in 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.
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 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 (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 Notes and Comments to the RNA isolation protocol (Note # 5) further eliminates the possibility of DNA contamination in RNA extracted by TRI REAGENT. 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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