



PRODUCT: TRI REAGENT® RT – RNA / DNA / PROTEIN ISOLATION REAGENT

Cat. No: RT 111

Storage: Store at room temperature.

PRODUCT DESCRIPTION

TRI Reagent® RT is an upgraded (patent pending) reagent for the single-step method of RNA isolation. Unlike the previous single-step methods, TRI Reagent® RT isolates RNA that is free of DNA contamination, as tested by 35-cycle PCR. Thus, no DNase treatment is necessary to use the isolated RNA in RT-PCR.

TRI Reagent® RT provides a reliable and cost-effective method of RNA isolation. TRI Reagent® RT combines phenol and guanidine thiocyanate in a mono-phase solution to facilitate effective inhibition of RNase activity. A biological sample is homogenized or lysed in TRI Reagent® RT and the homogenate is separated into aqueous and organic phases by bromoanisole addition and centrifugation. After phase separation, RNA remains in the aqueous phase while DNA and proteins are sequestered into the interphase and organic phase. RNA is precipitated from the aqueous phase by addition of isopropanol, washed with ethanol and solubilized. DNA and proteins can be isolated from the interphase and organic phase. The protocol for the RNA isolation is provided below and protocols for DNA and protein isolation are provided on our website at mrcgene.com.

TRI Reagent® RT isolates RNA from a wide variety of samples of human, animal, plant, yeast, bacterial and viral origin. RNA isolation is completed in less than 1 hour.

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

SPECIAL HANDLING PRECAUTIONS

TRI Reagent RT contains a poison (phenol) and an irritant (guanidine thiocyanate). Causes burns. CAN BE FATAL. When working with TRI Reagent RT **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.

PROTOCOL FOR ISOLATION OF RNA

The TRI Reagent RT procedure can be completed in 1 h and the isolated RNA is free of DNA contamination, as tested by 35-cycle PCR. TRI Reagent RT 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 RT-PCR, northern analysis, dot blot hybridization, poly A⁺ selection, in vitro translation, RNase protection assay and molecular cloning.

Reagents required but not supplied: 4-bromoanisole (BAN, Cat. No. BN 191), 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 RT.

The isolation is carried out at room temperature unless stated otherwise. The protocol includes the following steps:

1. **HOMOGENIZATION** – 1 ml TRI Reagent RT + 50 – 100 mg tissue, 5 – 10 x 10⁶ cells or 10 cm² of culture plate.
2. **PHASE SEPARATION** – homogenate + 50 µl bromoanisole, at 4 C.
3. **RNA PRECIPITATION** – 0.5 ml aqueous phase + 0.5 ml isopropanol.

4. RNA WASH – 1 ml 75% ethanol.

5. RNA SOLUBILIZATION – water or FORMAzol®.

1. HOMOGENIZATION

A. TISSUES. Homogenize tissue samples with TRI Reagent RT in a glass-Teflon or Polytron homogenizer. Use 50 – 100 mg tissue per 1 ml TRI Reagent RT. **Sample volume should not exceed 10% of the volume of TRI Reagent RT** used for homogenization. Tissues with high DNA content, such as spleen or liver, should be processed using 50 mg tissue / 1 ml of TRI Reagent RT.

B. CELLS. Cells grown in monolayer should be lysed directly in a culture dish. Pour off media, add TRI Reagent RT and pass the cell lysate several times through a pipette. Use 1 ml of TRI Reagent RT per 10 cm² of culture dish area. See also Note 3.

Cells grown in suspension should be sedimented first and then lysed in TRI Reagent RT by repetitive pipetting. Use 1.0 ml of the reagent per 5 – 10 x 10⁶ animal, plant or yeast cells or per 10⁷ bacterial cells.

Avoid washing cells before the addition of TRI Reagent RT as this may contribute to mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. PHASE SEPARATION

Supplement homogenate with 50 µl of bromoanisole per 1 ml of TRI Reagent RT used for homogenization. Cover the samples tightly and shake vigorously for 15 seconds. Centrifuge the resulting mixture at 12,000 g for 15 minutes at 4 C. Following centrifugation, the mixture separates into a lower, red phenol phase, the interphase, and the upper aqueous phase. RNA remains in the aqueous phase whereas DNA and proteins are in the interphase and organic phase.

It is important to separate phases in the cold (4 – 10 C). Centrifugation performed at elevated temperatures may sequester DNA into the aqueous phase. The use of bromoanisole for phase separation improves the quality of isolated RNA and eliminates toxic chloroform and bromochloropropane from the isolation protocol.

3. RNA PRECIPITATION

The resulting aqueous phase constitutes about 70% of the volume of TRI Reagent RT used for homogenization plus sample volume (mg = l). Collect 0.5 ml of the aqueous phase and transfer it to a fresh tube. During the collection, leave the remaining aqueous phase as an undisturbed layer to avoid collection of DNA from the interphase. Save the interphase and organic phase at 4 C or –20 C for subsequent isolation of DNA and proteins.

Precipitate RNA by mixing the aqueous phase with 0.5 ml of isopropanol. Store samples at room temperature for 5 – 10 minutes and centrifuge at 4,000 – 12,000 g for 5 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 1 ml of 75% ethanol by vortexing and subsequent centrifugation at 4,000 – 6,000 g for 5 minutes at 4 – 25 C. If the RNA pellet accumulates on the side of the tube or has a tendency to float, sediment the pellet at 12,000 g. *The ethanol wash can be repeated to improve 260/280 ratio of the isolated RNA.*

P>5. RNA SOLUBILIZATION

Remove the ethanol wash and add water to the RNA pellet. Avoid drying the pellet as this will decrease its solubility. Hydrate the samples in water for 5 – 10 minutes at room temperature, or if necessary, at 55 – 60 C. Dissolve RNA by vortexing or passing the solution through a pipette tip few times. Typically, amount of water used for solubilization should be sufficient to obtain RNA concentration ranging 0.5 – 2 µg/ml.

Water used for the RNA solubilization should be RNase-free. Due to the higher purity, it is easier to dissolve RNA isolated by TRI Reagent RT as compared to previous single-step methods.

Alternatively, dissolve RNA in FORMAzol® (MRC Cat. No. FO 121). FORMAzol is a convenient medium to store RNA samples for a few days at room temperature or for long term storage at – 20 C. It protects RNA from degradation due to accidental contamination during storage and handling of samples. Before use in RT-PCR, RNA samples dissolved in FORMAzol have to be precipitated with ethanol or diluted at least 50 times in an RT mix.

6. RESULTS

The Bioanalyzer test or agarose gel electrophoresis of the isolated RNA shows predominant bands of small (2 kb) and large (5 kb) ribosomal RNA, low molecular weight (0.1 – 0.3 kb) RNA, and in some preparations discrete bands of high molecular weight (7 – 15 kb) RNA. The RNA Integrity Number (RIN) of the isolated RNA is > 8.5.

The isolated RNA is free of DNA and proteins and has a 260/280 ratio 1.6 – 1.9. 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 (g RNA/10⁶ cells): epithelial cells, 8 – 15 µg; fibroblasts, 5 – 7 µg.

NOTES

1. To facilitate isolation of RNA from small samples (< 10⁶ cells or < 10 mg tissue) perform homogenization or lysis in 0.8 ml of TRI Reagent RT supplemented with 2 – 3 µl of Polyacryl CarrierTM (Cat. No. PC 152). Next, add bromoanisole and proceed with the phase separation and other steps of isolation as described above.
2. After homogenization and before bromoanisole addition, samples can be stored at –20 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 –20 C for at least one year.

3. For cells grown in monolayer, use the amount of TRI Reagent RT based on the area of a culture dish and not on cell number. Using an insufficient amount of TRI Reagent RT may result in DNA contamination of the isolated RNA.
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 centrifugation 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 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 and poly A⁺ RNA isolation Application Notes.
7. Whole blood and serum should be processed with TRI Reagent RT-BLOOD (RB 211). Other liquid samples should be processed with TRI Reagent RT-LIQUID SAMPLES (RL 311).

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 -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 RT 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. Also, low molecular weight RNA (>300 b) remains partially soluble.

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.

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Use of TRI Reagent® RT can be cited by referring to this brochure: TRI Reagent® RT – RNA, DNA, protein isolation reagent. Manufacturer's protocol (2008), Molecular Research Center, Inc. Cincinnati, OH or to reference 1.

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II. ISOLATION OF DNA BY TRI REAGENT® RT

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 RT 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 RT 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:

1. DNA PRECIPITATION – phenol phase and interphase + 0.3 ml ethanol (per 1 ml TRI Reagent RT).

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 RT.

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 RT 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 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 RT 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 RT), 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 the 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 ensures 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 (g DNA/mg tissue): liver, kidney, 3 – 4 µg; skeletal muscles, brain, placenta 2 – 3 µg. B) Cultured human, rat and mouse cells 5 – 7 µg DNA/10⁶ 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 below). 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 below). 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 g 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)	F
8.4 86	7.8 117	7.2
8.2 93	7.5 159	7.0
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 TM(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 – 5 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 mM guanidine thiocyanate, 50 mM sodium citrate and 1 mM 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 RT 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 RT 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 – 5 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® RT

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 RT.

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 1 and 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 RT protein extracts prepared from rat tissues yield 50 – 110 g 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

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 (RNA Isolation, Step 3), the RNA pellet can be dissolved in a poly A⁺ binding buffer and poly A⁺ RNA selection can be performed on oligo – dT columns according to a standard protocol of Aviv and Leder (Proc Natl Acad Sci USA, 1972, 69, 1408–1412). For the best results use MRC oligo-dT columns (Cat. No. OT 125).

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35, 450–456.

Use of TRI Reagent® RT can be cited by referring to this brochure: TRI Reagent® RT – RNA, DNA, protein isolation reagent. Manufacturer's protocol (2008), Molecular Research Center, Inc. Cincinnati, OH or to reference 1.

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