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Product Information

TRI Reagent® LS
for fluid samples

Catalog Number **T3934**
Store at room temperature.

TECHNICAL BULLETIN

Product Description
TRI Reagent LS is a quick and convenient reagent for use in the simultaneous isolation of RNA, DNA, and protein from liquid samples of human, animal, plant, yeast, bacterial, and viral origin. TRI Reagent LS is **not** suitable for use with whole blood; for whole blood samples, TRI Reagent BD (Catalog Number T3809) is recommended.

A convenient single-step liquid phase separation results in the simultaneous isolation of RNA, DNA, and protein.[1] This procedure is an adaptation of the single-step method reported by Chomczynski and Sacchi[2] for total RNA isolation, and permits fast and efficient processing of liquid samples. TRI Reagent LS performs well with large or small sample volumes, and many samples can be simultaneously extracted.

TRI Reagent LS is a mixture of guanidine thiocyanate and phenol in a monophase solution. When a biological sample is homogenized or lysed with it, and chloroform or 1-bromo-3-chloropropane is added, the mixture separates into 3 phases: an aqueous phase containing RNA, the interphase containing DNA, and an organic phase containing proteins. Each component can then be isolated after separating the phases. 0.75 ml of TRI Reagent LS processes 0.25 ml of a liquid sample such as amniotic fluid.

This is one of the most effective methods for isolating total RNA from fresh samples in only one hour. The procedure is very effective for isolating RNA molecules of all types from 0.1–15 kb in length. The resulting RNA is intact with little or no contaminating DNA and protein. This RNA can be used for Northern blots, mRNA isolation, *in vitro* translation, RNase protection assay, cloning, and polymerase chain reaction (PCR).

The DNA is in the interphase and phenol phase, which forms after the addition of chloroform or 1-bromo-3-chloropropane to the TRI Reagent LS in Sample Preparation, step 2. After precipitation and multiple washes, the DNA is dissolved in 8 mM NaOH. The solution is neutralized and the DNA is ready for analysis. The resulting DNA is suitable for PCR, restriction enzyme digestion, and Southern blotting.

After precipitating the DNA with ethanol (DNA Isolation, step 1) the proteins can be removed from the phenol-ethanol supernatant. The isolated material can be probed for specific proteins by Western blotting.[1]

Reagents Required but Not Provided
(Catalog numbers are given where appropriate)

RNA Isolation:
- Chloroform, Catalog Number C2432, or 1-Bromo-3-chloropropane, Catalog Number B9673
- Isopropanol, Catalog Number I9516
- 75% ethanol
- 1 mM sodium phosphate, Catalog Number S3264, pH 8.2, 0.5% SDS solution, Catalog Number L4522, diluted 20-fold, formamide, **or** diethylpyrocarbonate-treated water

DNA Isolation:
- 8 mM NaOH
- 0.1 M trisodium citrate, 10% ethanol solution
- Absolute ethanol
- 75% ethanol
- EDTA

Protein Isolation:
- Isopropanol, Catalog Number I9516
- Absolute ethanol, Catalog Number E7023
- 95% Ethanol, Catalog Number E7148
- 1% SDS, Catalog Number L4522, diluted 10-fold
- 0.3 M Guanidine hydrochloride, Catalog Number G3272, in 95% ethanol

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the product at room temperature.

Procedures
Sample Preparation

1A. 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 $5–10 \times 10^6$ cells.

1B. Tissue Suspensions:
Homogenize 0.25 ml sample with 0.75 ml of TRI Reagent LS in a glass-PTFE or Polytron® homogenizer.
Notes:
a. 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 always be 3:1.
b. For isolation of RNA from cells grown in monolayer add 0.3–0.4 ml of TRI Reagent LS per 10^2 cm area of a culture dish. 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 continue as described.
c. An additional step may be required for samples with a high content of polysaccharides, fat, proteins, or extracellular material. Following homogenization, remove insoluble material from the homogenate by centrifugation at $12,000 \times g$ for 10 minutes at 2-8 °C. The resulting pellet contains polysaccharides, extracellular membranes, and high molecular mass DNA, while the supernatant contains the 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. High molecular mass DNA can be recovered from the pellet by following DNA isolation, steps 2 and 3.
d. After the cells have been homogenized or lysed in TRI Reagent LS, samples can be stored at –70 °C for up to one month.

2. Phase Separation: To ensure complete dissociation of nucleoprotein complexes, allow samples to stand for 5 minutes at room temperature. Add 0.1 ml of 1-bromo-3-chloropropane or 0.2 ml of chloroform (see Phase Separation, notes a and b) per 0.75 ml of TRI Reagent LS used. Cover the sample tightly, shake vigorously for 15 seconds, and allow to stand for 2–15 minutes at room temperature. Centrifuge the resulting mixture at $12,000 \times g$ for 15 minutes at 2-8 °C. Centrifugation separates the mixture into 3 phases: a lower red organic phase (containing protein), an interphase (containing DNA), and a colorless upper aqueous phase (containing RNA). The volume of the aqueous phase is about 70% of the volume of TRI Reagent LS used for homogenization.
Notes:
a. 1-Bromo-3-chloropropane is less toxic than chloroform and its use for phase separation decreases the possibility of contaminating RNA with DNA.[3]
b. The chloroform used for phase separation should not contain isoamyl alcohol or other additives.
c. For isolation of poly A^+ fraction from the aqueous phase see Appendix I.

RNA Isolation
Store the interphase and organic phase at 2-8 °C for subsequent isolation of the DNA and proteins.

1. RNA Precipitation: Transfer the aqueous phase to a fresh tube and add 0.5 ml of isopropanol per 0.75 ml of TRI Reagent LS used for the initial homogenization. Allow the sample to stand for 5–10 minutes at room temperature. Centrifuge at $12,000 \times g$ for 8 minutes at 4–25 °C. The RNA precipitate will form a pellet on the side and bottom of the tube.
2. RNA Wash: Remove the supernatant and wash the RNA pellet by adding a minimum of 1 ml of 75% ethanol per 0.75 ml of TRI Reagent LS used for the initial homogenization. Vortex the sample and then centrifuge at $7,500 \times g$ for 5 minutes at 4–25 °C.
Notes:
a. If the RNA pellet floats or accumulates on a side of the tube, perform the wash in 75% ethanol at $12,000 \times g$.
b. Samples can be stored in ethanol at 2-8 °C for at least 1 week and up to 1 year at –20 °C.

3. RNA Solubilization: Briefly dry the RNA pellet for 5–10 minutes by air-drying or under a vacuum. Do not let the RNA pellet dry completely, as this will greatly decrease its solubility. Do not dry the RNA pellet by centrifugation under vacuum (SpeedVac®). Add an appropriate volume of 1 mM sodium phosphate, pH 8.2, formamide, water, or 0.5% SDS solution to the RNA pellet. SDS and formamide may interfere with subsequent reactions. Formamide inhibits reverse transcriptase and is not recommended for solubilization of RNA used for RT-PCR. To facilitate dissolution, mix by repeated pipetting with a micropipette and incubating at 55–60 °C for 10–15 minutes.
   Notes:
   a. Final preparation of RNA is free of DNA and proteins. It should have a 260/280 ratio of ≥1.7.
   b. Ethidium bromide staining of RNA in agarose gels visualizes two predominant bands of small (2 kb) and large (5 kb) ribosomal RNA, low molecular mass (0.1–0.3 kb) RNA, and discrete bands of high molecular mass (7–15 kb) RNA.

DNA Isolation

1. DNA Precipitation: Carefully remove the remaining aqueous phase overlaying the interphase and discard. To precipitate the DNA from the interphase and organic phase, add 0.3 ml of 100% ethanol per 0.75 ml of TRI Reagent LS used in Sample Preparation, step 1. Mix by inversion and allow to stand for 2–3 minutes. Centrifuge at 2,000 × *g* for 5 minutes at 2-8 °C.
   Note: Removal of the remaining aqueous phase before DNA precipitation is a critical step for the quality of the isolated DNA.
2. DNA Wash: Remove the supernatant and save at 2-8 °C for protein isolation. Wash the DNA pellet twice in a 0.1 M trisodium citrate, 10% ethanol solution. During each wash, allow the DNA pellet to stand with occasional mixing for at least 30 minutes. Centrifuge at 2,000 × *g* for 5 minutes at 2-8 °C. Resuspend the DNA pellet in 75% ethanol (1.5–2 ml for each ml of TRI Reagent LS) and allow to stand for 10–20 minutes at room temperature. Centrifuge at 2,000 × *g* for 5 minutes at 2-8 °C. This ethanol wash removes pinkish color from the DNA pellet.
   Notes:
   a. **Important**: Do not reduce the time samples remain in the washing solution. Thirty minutes is the absolute minimum time for efficient removal of phenol from the DNA.
   b. If pellet contains >200 μg of DNA or large amounts of non-DNA material, an additional wash in 0.1 M trisodium citrate, 10% ethanol solution is required.
   c. Samples suspended in 75% ethanol can be stored at 2-8 °C for several months.
3. DNA Solubilization: Air dry the DNA pellet for 5–15 minutes at room temperature. Dissolve the DNA pellet in 8 mM NaOH by repeated slow pipetting with a micropipette. Add sufficient 8 mM NaOH for a final DNA concentration of 0.2–0.3 μg/μl (typically, add 0.3-0.6 ml to the DNA isolated from 10^7 cells). This mild alkaline solution assures complete dissolution of the DNA pellet. Centrifuge at 12,000 × *g* for 10 minutes to remove any insoluble material and transfer the supernatant to a new tube.
   Notes:
   a. A viscous supernatant indicates the presence of high molecular mass DNA.
   b. The size of the DNA will depend on the force exerted during homogenization. Avoid using a Polytron homogenizer.
   c. Samples dissolved in 8 mM NaOH can be stored at 2-8 °C overnight. For long term storage, adjust the pH to between 7 and 8, and supplement with EDTA (final concentration 1 mM).
   d. To determine DNA concentration, remove an aliquot, dilute with water, and measure the A_{260}. For double stranded DNA, 1 A_{260} unit/ml = 50 μg/ml.
   e. To calculate cell number, assume the amount of DNA for 10^6 diploid cells of human, rat, and mouse equals 7.1 μg, 6.5 μg, and 5.8 μg respectively.[4]
   f. A preparation of DNA isolated from tissue suspension typically contains 70% of 60–100 kb DNA and 30% of 20 kb DNA. The isolated DNA is free of RNA and proteins, and has a 260/280 ratio >1.7.

To Amplify DNA by PCR:
After dissolving in 8 mM NaOH, adjust to pH 8.4 using HEPES (add 66 μl of 0.1 M HEPES, free acid/ml of DNA solution). Add sample (generally 0.1–1 μg) to PCR mix and follow PCR protocol.

To Digest DNA with Restriction Enzymes

Adjust the pH of the DNA solution to that needed for the restriction enzyme digestion using HEPES, or dialyze samples against 1 mM EDTA, pH 7–8. Allow the restriction enzyme digestion to continue for 3–24 hours under optimal conditions. It is recommended that 3–5 units of enzyme be used per 1 μg of DNA. Typically, 80–90% of the DNA is digested.

Protein Isolation

1. Protein Precipitation: Precipitate proteins (see note, step 3) from the phenol-ethanol supernatant (DNA Isolation, step 2) with 1.5 ml of isopropanol per 0.75 ml of TRI Reagent LS used in the initial Sample Preparation, step 1. Allow samples to stand for at least 10 minutes at room temperature. Centrifuge at 12,000 × *g* for 10 minutes at 2-8 °C.
2. Protein Wash: Discard supernatant and wash the pellet 3 times in 0.3 M guanidine hydrochloride/ 95% ethanol solution, using 2 ml per 0.75 ml of TRI Reagent LS used in the initial sample preparation. During each wash, store samples in wash solution for 20 minutes at room temperature. Centrifuge at 7,500 × *g* for 5 minutes at 2-8 °C. After the 3 washes, add 2 ml of 100% ethanol and vortex the protein pellet. Allow to stand for 20 minutes at room temperature. Centrifuge at 7,500 × *g* for 5 minutes at 2-8 °C.
   Note: Protein samples suspended in 0.3 M guanidine hydrochloride/95% ethanol solution or 100% ethanol can be stored for 1 month at 2-8 °C or 1 year at –20 °C.
3. Protein Solubilization: Air dry the protein pellet for 5–15 minutes. Dissolve pellet in 1% SDS aided by working the plunger of micropipette with tip in the solution. Some protein pellets may require incubation at 50 °C for complete solubilization. Remove any insoluble material by centrifugation at 10,000 × *g* for 10 minutes at 2-8 °C. Transfer supernatant to a new tube. The protein solution should be used immediately for Western blotting or stored at –20 °C.
   Note: For some samples, the protein pellet may be difficult to dissolve in 1% SDS (Protein Isolation, step 3). Use this alternate procedure to correct the problem:
   a. Dialyze the phenol-ethanol supernatant against 3 changes of 0.1% SDS at 2-8 °C.
   b. Centrifuge the dialysate at 10,000 × *g* for 10 minutes at 2-8 °C.
   c. The clear supernatant contains protein that is suitable for use in Western blotting procedures.

Troubleshooting Guide

1. RNA Isolation:
   A. Low yield may be due to:
      - incomplete homogenization or lysis of samples.
      - the final RNA pellet may not have been completely dissolved.
   B. If the A_{260}/A_{280} ratio is <1.65:
      - the amount of reagent used in lysis may have been too great
      - samples may not have been allowed to stand at room temperature for 5 minutes after homogenization.
      - there may have been contamination of the aqueous phase with phenol phase
      - the isolated RNA may contain some protein, which can be removed by adding 1 ml of TRI Reagent LS per 100 μl of RNA solution and repeating the sample preparation and RNA isolation steps.
      - the final RNA pellet may not have been completely dissolved
      - the pH of the DEPC-treated water may have been too low; 1 mM sodium phosphate buffer, pH 8.0-8.5, should be used to solubilize the RNA.[6]
   C. If there is degradation of the RNA:
      - samples were not immediately processed or frozen after collection.
      - the samples used for isolation or the isolated RNA preparations may have been stored at –20 °C instead of –70 °C as specified in the procedure.
      - cells were dispersed by trypsin digestion.
      - aqueous solutions or tubes used for procedure may not have been RNase free
      - formaldehyde used for the agarose-gel electrophoresis may have had a pH value <3.5.
   D. If there is DNA contamination:
      - the volume of reagent used for sample lysis or homogenization may have been too small.
      - samples used for the isolation contained organic solvents (ethanol or DMSO), strong buffers, or alkaline solution.

2. DNA Isolation:
   A. Low yield may be due to:
      - the final DNA pellet may not have been completely dissolved.
   B. If the A_{260}/A_{280} ratio is <1.70:
      - phenol was not sufficiently removed from the DNA preparation. Wash the DNA pellet an additional time with the 0.1 M trisodium citrate, 10% ethanol solution.
   C. If there is degradation of the DNA:
      - the samples may not have been immediately processed or frozen after collection.
      - samples used for isolation were stored at –20 °C instead of at –70 °C as specified in the procedure.
      - samples were homogenized with a Polytron or other high speed homogenizer.
   D. If there is RNA contamination:
      - there may have been too much aqueous phase remaining with the organic phase and interphase.
      - the DNA pellet was not sufficiently washed with 0.1 M trisodium citrate, 10% ethanol solution.
3. Protein Isolation:
   A. If there is low yield:
      - samples may have been incompletely lysed or homogenized.
      - the final protein pellet may not have been completely dissolved.
   B. If there is degradation of the protein:
      - the samples were not immediately processed or frozen after collection.
   C. If PAGE shows band deformation:
      - the protein pellet may not have been washed sufficiently.

Appendix

I. Isolation of Poly A^+ RNA

After the RNA has been precipitated with isopropanol (RNA Isolation, step 1), dissolve the pellet in poly A^+ binding buffer and pass through an oligo-dT cellulose (Catalog Number O3131) column to selectively remove mRNA according to the procedure of Aviv and Leder.[5]

II. Isolated RNA is to be used in RT-PCR

1. Modifying the procedure by performing the additional centrifugation step in the initial Sample Preparation, step 1B, note c further minimizes the possibility of DNA contamination in the RNA extracted by TRI Reagent LS.
2. A more complete evaporation of ethanol is required when RNA samples are to be used in RT-PCR. This is especially critical for small volume samples (5–20 μl), which may contain a relatively high level of ethanol if not adequately dried.

References

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