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Product Information

TRI Reagent® BD
For processing whole blood, plasma, or serum
Catalog Number **T3809**

Product Description

TRI Reagent BD is a quick and convenient reagent for use in the simultaneous isolation of RNA, DNA and protein from serum, plasma or whole blood. 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 blood derivatives. TRI REAGENT BD performs well with large or small sample volumes, and many samples can be simultaneously extracted.

TRI Reagent BD is a mixture of guanidine thiocyanate and phenol in a mono-phase solution. When a sample of blood derivatives is lysed with it, and chloroform or 1-bromo-3-chloropropane is added, the mixture separates into 3 phases: an aqueous phase containing the 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 BD processes 0.25 ml of blood derivatives.

This is one of the most effective methods for isolating total RNA and can be completed in only 1 hour starting with fresh cells. The procedure is very effective for isolating RNA molecules of all types from 0.1 to 15 kb in length. The resulting RNA is intact with little or no contaminating DNA or protein. This RNA can be used for Northern blots, mRNA isolation, *in vitro* translation, RNase protection assays, cloning and polymerase chain reaction (PCR)*.

The protocol for DNA isolation with TRI REAGENT BD is designed to overcome the heavy burden of proteins present in whole blood. The DNA is in the interphase which forms after the addition of chloroform to the TRI Reagent BD in step 2 of sample preparation.

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 (Step 1 of DNA Isolation), the proteins can be removed from the phenol-ethanol supernatant. The isolated material can be probed for specific proteins by immunoblotting.[1]

Items Required But Not Provided

RNA Isolation:

- Chloroform, Catalog No. C2432, or 1-Bromo-3-chloropropane, Catalog No. B9673
- Isopropanol, Catalog No. I9516
- 75% Ethanol
- 1 mM Sodium phosphate, Catalog No. S3264, pH 8.2, 0.5% SDS solution, Catalog No. L4522, diluted 20-fold, Formamide, or Diethylpyrocarbonate (DEPC)-treated water
- 5 N Acetic acid

DNA Isolation:

- 8 mM NaOH
- Absolute ethanol
- 95% Ethanol
- DNAzol® (available from Molecular Research Center, Inc.)
- 1 mM EDTA

Protein Isolation:

- Isopropanol, Catalog No. I9516
- Absolute ethanol
- 95% Ethanol
- 1% SDS
- 0.3 M Guanidine hydrochloride in 95% ethanol

Precautions
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
Store at room temperature.

I. Sample Preparation

1A. Serum:
Add 0.25 ml of serum to 0.75 ml of TRI Reagent BD. Close the tube and shake the solution by hand or vortex, ensuring that mixing is thorough.

1B. Whole Blood or Plasma:
Add 0.2 ml of whole blood or plasma to 0.75 ml of TRI Reagent BD supplemented with 20 µl of 5 N acetic acid per 0.2 ml of whole blood or plasma. Close the tube and shake the solution by hand or vortex, ensuring that mixing is thorough.

Notes:
a. Acetic acid can be added before or after mixing TRI Reagent BD with blood samples.
b. Prepare 5 N acetic acid by mixing 1 ml of glacial acetic acid (>99%) with 2.48 ml of water.
c. The ratio of the sample volume to the reagent volume should always be as indicated in the protocol above. A sample volume which is too large will result in DNA contamination, and a sample volume which is too small will lower the yield of RNA.
d. Samples can be stored for several months at –70 °C at this point.

2. Phase Separation: To ensure complete dissociation of nucleoprotein complexes, allow samples to stand for 5 minutes at room temperature. Add 0.1 ml 1-bromo-3-chloropropane or 0.2 ml of chloroform (see note below) per 0.75 ml of TRI Reagent BD used. Cover the sample tightly, shake vigorously for 15 seconds and allow to stand for 2-5 minutes at room temperature. Centrifuge the resulting mixture at 12,000 x g for 15 minutes at 4 °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).

Note:
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 the special note at the end of the bulletin.

II. RNA Isolation

Note: Store the interphase and organic phase at 4 °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 BD used for the initial lysis and mix. Allow the sample to stand for 5-10 minutes at room temperature. Centrifuge at 12,000 x 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 1 ml (minimum) of 75% ethanol per 0.75 ml of TRI Reagent BD. Vortex the sample and then centrifuge at 7,500 x g for 5 minutes at 4-25 °C.

Notes:
a. If the RNA pellets float, perform the wash with 75% ethanol at 12,000 x g.
b. Samples can be stored in ethanol at 4 °C for at least 1 week and up to 1 year at –20 °C.
c. If the isolation is performed in a tube greater than 2 ml, add 1 ml of 75% ethanol to the RNA pellet and transfer the RNA-ethanol suspension to a microcentrifuge tube. For the transfer, use a wide bore 1 ml pipette tip prepared by cutting 2-3 mm from the end of a plastic tip.

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.** Add an appropriate volume of 1 mM sodium phosphate , pH 8.2[6], 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 to be used for RT-PCR. To facilitate dissolution, mix by repeated pipetting with a micropipette 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. Typical yields from 1 ml of human whole blood: 15-20 μg.

Cellular RNA degrades rapidly when blood samples are stored at 4 °C. For isolation of undegraded RNA, samples should be processed immediately after collection or aliquoted at –70 °C with or without TRI REAGENT BD. Isolation of viral RNA can be performed with samples stored at 4 °C for several days.

III. 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 0.4 ml of 100% ethanol per 0.75 ml of TRI Reagent BD used in step 1 of Sample Preparation. Mix by inversion and centrifuge at 2,000 x g for 5 minutes at 4 °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 4°C for protein isolation. Add 0.25 ml of DNAzol and dissolve the DNA precipitate by agitating the tube. When the isolation is performed in large (>2 ml) tubes, transfer the DNA solution to a microcentrifuge tube.
3. DNA Precipitation: Precipitate the DNA by adding 0.125 ml of ethanol (100%) to 0.25 ml of DNAzol and allowing to stand for 2-5 minutes. Centrifuge at 2,000 x g for 3 minutes at 4 °C.
4. DNA Wash: Wash the DNA precipitate twice with 0.8-1 ml of 95% ethanol. Each time, suspend the precipitate by inverting the tubes. Centrifuge at 2,000 x g for 1-3 minutes. If the DNA forms a compact pellet, no centrifugation is necessary and the ethanol wash can be removed by decantation.
5. DNA Solubilization: Air dry the pellet by opening the tubes for 5-10 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 0.1-0.3 μg/μl (typically, add 0.1 ml to the DNA isolated from 1 ml of whole blood). This mild alkaline solution assures complete dissolution of the DNA pellet.

Notes:

a. Samples dissolved in 8 mM NaOH can be stored at 4 °C overnight. For long term storage, adjust the pH to between 7 and 8 and supplement with EDTA, (final concentration 1 mM).
b. 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.
c. 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]
d. Typical yields from human whole blood: 10-20 μg.
e. A preparation of DNA isolated from whole blood typically contains >80% of 60-100 kb DNA and <10% 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) per 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.

IV. Protein Isolation

1. Protein Precipitation: Precipitate proteins (see note below) from the phenol-ethanol supernatant (DNA Isolation, step 2) with 1.5 ml of isopropanol per 0.75 ml of TRI Reagent BD used in the initial sample preparation (step 1). Allow samples to stand for at least 10 minutes at room temperature. Centrifuge at 12,000 x g for 10 minutes at 4 °C.

2. Protein Wash: Discard supernatant and wash pellet 3 times in 0.3 M guanidine hydrochloride/95% ethanol solution, using 2 ml per 0.75 ml of TRI REAGENT BD used in the initial sample preparation. During each wash, store samples in wash solution for 20 minutes at room temperature. Centrifuge at 7,500 x g for 5 minutes at 4 °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 x g for 5 minutes at 4 °C.

Note: Protein samples suspended in 0.3 M guanidine hydrochloride/95% ethanol solution or 100% ethanol can be stored for 1 month at 4 °C or 1 year at –20 °C.

3. Protein Solubilization: Dry protein pellet under a vacuum for 5-10 minutes. Dissolve pellet in 1% SDS aided by working the plunger of micropipette with tip in the solution. Remove any insoluble material by centrifugation at 10,000 x g for 10 minutes at 4 °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 (step 3 above). Use this alternate procedure to correct the problem:

a. Dialyze the phenol-ethanol supernatant against 3 changes of 0.1% SDS at 4 °C.
b. Centrifuge the dialysate at 10,000 x g for 10 minutes at 4 °C.
c. The clear supernatant contains protein that is suitable for use in western blotting procedures.

Troubleshooting Guide

I RNA Isolation:

A. Low yield may be due to:
- incomplete lysis of samples.
- incomplete solution of the final RNA pellet.

B. If the A_{260}/A_{280} ratio is <1.65:
- the amount of reagent used in lysis may have been too great.
- there may have been contamination of the aqueous phase with phenol phase.
- the isolate RNA may contain some protein which can be removed by adding 1 ml of TRI REAGENT BD 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:
- the samples may not have been 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.
- 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 below 3.5.

D. If there is DNA contamination:
- the volume of reagent used for sample lysis may have been too small.

II. DNA Isolation:
   A. Low yield may be due to:
      - incomplete lysis of samples.
      - incomplete solubilization of the final DNA pellet.
   B. If the A_{260}/A_{280} ratio is <1.70:
      - incomplete solubilization of the final DNA pellet.
      - if insoluble particles remain in the DNA preparation, remove the particles by centrifugation at 10,000 x g for 5 minutes at 4°C.
   C. If there is degradation of the DNA:
      - the samples may not have been immediately processed or frozen after collection.
      - the blood samples were stored at room temperature.
      - too much mechanical force was applied during the procedure.
   D. If there is RNA contamination:
      - there may have been too much aqueous phase remaining with the organic phase and interphase.

III. Protein Isolation:
   A. Low yield may be due to:
      - incomplete lysis of samples.
      - the final protein pellet may not have been completely dissolved.
   B. If there is degradation of the protein:
      - the samples may not have been immediately processed or frozen after collection.
   C. If PAGE shows band deformation:
      - the protein pellet may not have been washed sufficiently.

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 No. O3131, column to selectively remove mRNA according to the procedure of Aviv and Leder.[5]

If Isolated RNA Is to Be Used in RT-PCR

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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