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A Geno Technology, Inc. (USA) brand name

Tri-Xtract™

For the Simultaneous Isolation of RNA, DNA & Protein

(Cat. # 786-652, 786-653)

INTRODUCTION

Tri-Xtract™ is a convenient, ready-to-use reagent designed for the isolation of total RNA that is free from protein and DNA contamination. The isolated RNA is suitable for Northern blots, dot blot hybridization, in-vitro translation, RNase protection assays and poly (A^+) selection.

Tri-Xtract™ is a monophasic solution of phenol and guanidine thiocyanate that maintains the integrity of the RNA during cell disruption and homogenization, the addition of chloroform results in the separation of the homogenate into aqueous and organic phases. RNA partitions to the aqueous phase, DNA to the interphase, and proteins to the organic phase. The total RNA is recovered by isopropyl alcohol precipitation. The DNA and proteins can also be recovered by sequential precipitation from the organic phase.

Tri-Xtract™ is suitable for the isolation of total RNA, including mRNA, hnRNA, ribosomal RNA and tRNA, from small quantities of tissues (50-100mg) and cells (5×10^6) from human, animal, plant, yeast, bacterial and viral origin. Total RNA is isolated in under an hour and the subsequent isolation of DNA and protein in less than three hours.

ITEM(S) SUPPLIED

KIT COMPONENTS	Cat. # 786-652	Cat. # 786-653
Tri-Xtract™	100ml	2 x 100ml

STORAGE CONDITIONS

Tri-Xtract™ is shipped at ambient temperature. Upon arrival, store at 4°C, protected from light.

WARNING

- Product contains phenol that is highly toxic on contact with skin and if swallowed, causes chemical burns. Upon contact with skin, wash immediately with plenty of detergent and water and seek medical advice. Read the MSDS for toxicity details and other information.
- When using Tri-Xtract™ always wear gloves, eye protection and lab coats. Use in a fume hood.

PRECAUTIONS

Follow these guidelines to avoid RNAse contamination:

- Always wear gloves.
- Use sterile, disposable plastics and pipettes reserved for RNA work. Swab down equipment and gloves with an RNase decontaminator. We recommend *RNaseOUT*™ (Cat. # 786-70).
- Use DEPC treated water for subsequent purification of RNA, following Tri-Xtract™ treatment. DEPC-treated water is available in 100, 500 and 1000ml sizes (Cat. # 786-117, 786-118, 786-109).

ADDITIONAL ITEMS REQUIRED

For Total RNA

- Chloroform
- Isopropyl alcohol
- DEPC Treated water
- 70% Ethanol in DEPC-treated water

For DNA

- Ethanol
- 70% ethanol
- 8mM NaOH
- 0.1M Sodium citrate in 10% ethanol

For Protein

- Isopropyl alcohol
- Ethanol
- 1% SDS
- Guanidine hydrochloride (0.3M) in 95% ethanol

RECOMMENDED VOLUMES OF TRI-XTRACT™

Sample	**Tri-Xtract™ Volume (ml)**
$10cm^2$ Adherent Cells	1
10^7 Suspension Cells	1-2
100µl White Cells	2
50-100mg Soft Tissue	1
50-100mg Hard Tissue (Liver, Spleen, Bone Cartilage)	2
15-100mg Plant Tissue	1

PROTOCOL FOR RNA ISOLATION

For optimal RNA isolation, always use fresh biological samples.

1. ***Cell/ Tissue Homogenization***
   a. ***Tissues:*** Add 1ml Tri-Xtract™ for every 50-100mg tissue and homogenize with a glass, Teflon® or power homogenizer. Homogenization can be significantly improved by grinding in liquid nitrogen. Ensure the sample volume is less than 10% of the Tri-Xtract™ reagent.
   b. ***Adherent Cells:*** Add 1ml Tri-Xtract™ directly to adherent cells on a 3.5cm tissue culture dish and pipette up and down to lyse. Use 1ml Tri-Xtract™ for every $10cm^2$ cell surface area to ensure DNA and protein free RNA.
   c. ***Cells in suspension:*** Collect the cells by centrifugation and lyse 5-10 x 10^6 animal, plant or yeast cells or 1 x 10^7 bacterial cells with 1ml Tri-Xtract™. Pipette up and down to lyse the cells. Avoid washing cells prior to Tri-Xtract™ treatment to maintain mRNA. Some yeast and bacterial cells may require homogenization for complete lysis.

OPTIONAL*: The versatility of Tri-Xtract™ allows for RNA isolation from a variety of tissues. For tissues or cells high in polysaccharides, extracellular material and/or fats, we recommend an additional purification step. Following homogenization, centrifuge at 12,000 x g for 10 minutes at 4°C to pellet the insoluble material, which includes polysaccharides, cell debris and high molecular weight DNA. The RNA is in the supernatant. For tissues with a high fat content, the fats form a top layer after centrifugation that is easily removed with a pipette. Transfer the clear supernatant to a fresh tube and continue.*

2. Incubate the samples for 5 minutes at room temperature.
   NOTE*: The samples can be stored at -80°C for at least one month at this point.*
3. Add 200µl chloroform per 1ml Tri-Xtract™. Securely cap the tubes and vigorously shake the tubes for 15 seconds.
4. Incubate at room temperature for a further 3 minutes.
5. Centrifuge the tubes at a maximum 12,000xg for 15 minutes at 4°C.
6. Following separation, a lower pink, phenol-chloroform phase, an interphase, and a colorless, upper, aqueous phase are visible. The RNA is exclusively in the aqueous, upper phase. The volume of the aqueous phase is ~60% the volume of the Tri-Xtract™ reagent used.
7. Transfer the aqueous phase to a fresh tube and save the organic phase for DNA and protein isolation.
8. Add 500µl isopropyl alcohol for every 1ml Tri-Xtract™ originally used. Incubate at room temperature for 10 minutes and centrifuge at a maximum 12,000xg for 10 minutes at 4°C. A gel like pellet of RNA forms on the side and bottom of the tube.
   NOTE*: For samples high in proteoglycan and polysaccharide contamination, add 300µl isopropanol to the aqueous phase, followed by 300µl 0.8M sodium citrate and 1.2M NaCl for every 1ml Tri-Xtract™ originally used. Mix and incubate at room*

temperature for 5-10 minutes, then centrifuge at 12,000 x g for 8 minutes. Continue with step 9.

9. Remove the supernatant and wash the RNA pellet with 1ml 70% ethanol for every 1ml Tri-Xtract™ originally used.
   NOTE: *The samples can be stored at 4°C for at least one week or at -20°C for a least one year in 70%ethanol.*
10. Vortex to mix and centrifuge at 7,500 x g for 5 minutes at 4°C. Discard the supernatant wash.
11. Air dry the RNA pellet for 5-10 minutes, ensuring that it does not completely dry out. Dissolve the RNA pellet in an appropriate volume of prewarmed (55-60°C) RNase-free water by pipetting up and down. To aid the process, incubate at 55-60°C for 10 minutes.

PROTOCOL FOR DNA ISOLATION

1. The DNA is located in the interphase and the organic phenol phase. Ensure all the overlying aqueous phase is removed from the interphase. *If aqueous phase is not completely removed the quality of the recovered DNA will be affected.*
   NOTE: *The interphase and organic phase can be stored overnight at 4°C*
2. Add 300µl 100% ethanol for every 1ml Tri-Xtract™ used in the initial extraction.
3. Mix the samples by inverting the tube 5 times.
4. Incubate at room temperature for 3 minutes.
5. Pellet the precipitated DNA by centrifugation at a maximum 2,000g for 5 minutes at 4°C.
6. Carefully, remove the phenol/ethanol supernatant to a clean tube. If desired, this can be used for the protein isolation. Store at 4°C overnight.
7. Wash the DNA pellet with 1ml 0.1M sodium citrate in 10% ethanol per 1ml Tri-Xtract™. Incubate at room temperature for 30 minutes, with periodic mixing, and then centrifuge at 2,000g for 5 minutes at 4°C. Repeat wash step once. *If the DNA pellet is large (>200µg DNA) a third wash is recommended.*
   NOTE: *Do not shorten wash incubation times to avoid a phenol contamination.*
8. Suspend the DNA pellet in 1.5ml 70% ethanol per 1ml Tri-Xtract™ and incubate at room temperature for 10 minutes, before centrifuging at 2,000g for 5 minutes at 4°C.
9. Completely remove the ethanol wash with a pipette tip and allow the DNA pellet to air dry for up to 15 minutes.
10. Resuspend the DNA in 200-500µl 8mM NaOH to give a final concentration of ~0.3µg/µl. Following resuspension there may be some insoluble, gel-like material, consisting of membranes, remove by centrifugation at 12,000g for 10 minutes.
11. The solubilized DNA can be stored overnight at 4°C, however for longer storage adjust the pH to pH7-8 with HEPES buffer supplemented with 1mM EDTA. Store at 4°C or -20°C.
12. The isolated DNA can be neutralized and used for restriction digestion, PCR and Southern blotting. Also full recovery of DNA permits the determination of the DNA content in analyzed samples.
    a. For quantitation, dilute with a buffer with pH>7.5.
    b. For PCR Amplification, adjust pH to 8.4 with HEPES, as indicated below.
    c. For restriction analysis, adjust pH to required value.

Adjustment of pH in DNA Samples Solubilized with 8mM NaOH	
For 1ml of 8mM NaOH use the following amounts of HEPES (free acid)	
Final pH Required	**0.1M HEPES (µl)**
8.4	86
8.2	93
8.0	101
7.8	117
7.5	159
-	**1M HEPES (µl)**
7.2	23
7.0	32

PROTOCOL FOR PROTEIN ISOLATION

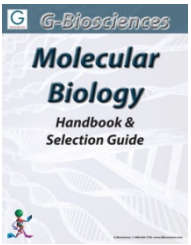
1. Precipitate the proteins from the phenol/ethanol supernatant (Protocol for DNA Isolation, step 6). Add 1.5ml isopropyl alcohol per 1ml Tri-Xtract™. Incubate samples at room temperature for 10 minutes and pellet the proteins by centrifugation at 12,000g for 10 minutes at 4°C.
2. Add 2ml 0.3M guanidine.HCl in 95% ethanol per 1ml Tri-Xtract™. Incubate at room temperature for 15 minutes and then centrifuge at 7,500g for 5 minutes at 4°C. Repeat this step two more times.
3. Add 2ml ethanol to the pellet, vortex and incubate at room temperature for 20 minutes. Centrifuge at 7,500g for 5 minutes at 4°C to pellet the protein.
4. Remove the ethanol and air dry the pellet for 15-30 minutes. Dissolve the pellet in 1% SDS. To aid the dissolving the pellet, incubate at 50°C. The insoluble material can be removed by centrifugation at 10,000g for 10 minutes. The resulting supernatant is ready for Western blot analysis.

TROUBLESHOOTING

RNA Isolation	
Low Yield	• Incomplete homogenization or lysis of sample • Incomplete solubilization of final RNA pellet
Poor Purity (260/280 ratio <1.6)	• Tri-Xtract™ volume too low for homogenization • Acidic water/buffer used for absorbance measurement • Contamination with phenol phase • Incomplete solubilization of final RNA pellet
RNA Degradation	• Tissue was not fresh or frozen prior to solubilization • Incorrect storage of samples or isolated RNA, store at -70°C • Trypsin was used to release adherent cells • RNase contamination, use RNaseOUT™ (Cat. # 786-70) to remove RNase from equipment
DNA Contamination	• Tri-Xtract™ volume too low for homogenization • Samples for isolation had organic solvents, strong buffers and/or alkaline solutions
DNA Isolation	
Low Yield	• Incomplete homogenization or lysis of sample • Incomplete solubilization of final DNA pellet
Poor Purity (260/280 ratio <1.7)	• Contamination by phenol • Acidic water/buffer used for absorbance measurement
DNA Degradation	• Tissue was not fresh or frozen prior to solubilization • Homogenization was too vigorous, avoid Polytron or high speed homogenization
RNA Contamination	• Too much aqueous phase carried over • Insufficient washing of DNA pellet in 0.1M sodium citrate in 10% ethanol
Protein isolation	
Low Yield	• Incomplete homogenization or lysis of sample • Incomplete solubilization of final DNA pellet
Protein Degradation	• Tissue was not fresh or frozen prior to solubilization
Poor Band resolution by PAGE	• Insufficient washing of protein pellet • Use PAGE-*Perfect*™ (Cat. # 786-123) to ensure high quality protein resolution

RELATED PRODUCTS

Download our Molecular Biology Handbook.



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