TriPure Isolation Reagent

Clear, red solution; ready-to-use

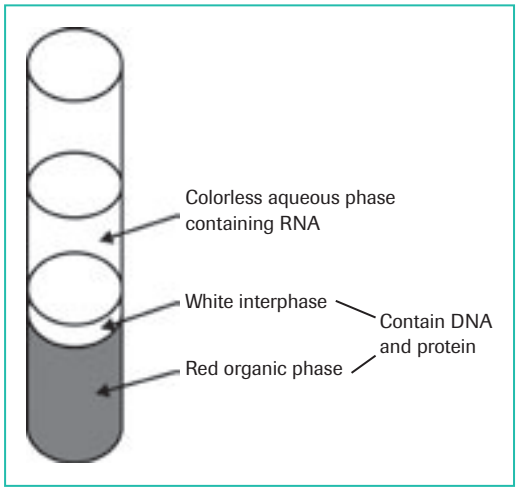
Cat. No. 11 667 157 001 (50 ml)
Cat. No. 11 667 165 001 (250 ml)

Principle	During a one-step sample homogenization/lysis procedure, the TriPure Isolation Reagent disrupts cells and denatures endogenous nucleases. After chloroform is added to the extract, the mixture is centrifuged and separates into three phases: a colorless aqueous (upper) phase, a white interphase and a red organic (lower) phase. The phases may then be separated and alcohol precipitation used to recover RNA (from the colorless aqueous phase), DNA and protein (from the interphase and red organic phase).
Starting material	• Cultured cells (research samples) • Fresh or frozen animal tissue (research samples) • Human leukocytes (research samples) • Bacterial cell suspensions • Yeast spheroplasts • Plant spheroplasts
Application	• Preparation of total RNA, genomic DNA, and protein from a single biological sample • DNA-free total RNA may be used for Northern blots, *in vitro* translation, RNase protection assays, cDNA synthesis, or RT-PCR • RNA-free DNA may be used for PCR, restriction analysis, Southern blots, and cloning • Denatured protein may be used for SDS-PAGE and Western blots
Time required	• Total time: approx. 2.5 h for RNA isolation • Hands-on time: approx. 25 min for RNA isolation
Results	• Yields vary depending on starting material (See the table under Part IV of "How to use the reagent" in this article) • A_{260}/A_{280} of RNA = 1.6 – 2.0 • A_{260}/A_{280} of DNA >1.7
Benefits	• **Saves time**, because the RNA isolation procedure requires only 1 h • **Easy to use**, because the red dye in the reagent simplifies identification of different phases • **Adapts easily to needs of specific laboratories**, because the reagent can be used with a wide variety of starting samples • **Simplifies isolation protocols**, because a single reagent can be used to isolate DNA-free RNA, RNA-free DNA, and protein for a variety of applications (see above) • **Increases yield of intact RNA**, because the reagent provides an immediate chaotropic denaturing environment that eliminates endogenous RNase activity

How to use the reagent

I. Flow diagram

(see page 159)



II. Reagent contents

TriPure Isolation Reagent is a clear, red, monophasic solution of phenol and guanidine thiocyanate, pH 4. It is ready to use as supplied.

III. Additional materials needed

For the extraction and phase separation protocol

- Sterile, disposable polypropylene tubes that can withstand 12,000 x *g* in the presence of TriPure Isolation Reagent and chloroform
- Chloroform (free of all additives such as isoamyl alcohol)

For RNA isolation

- Isopropanol
- 75% ethanol
- Diethylpyrocarbonate (DEPC)-treated, RNase-free water or DEPC-treated 0.5% SDS

For DNA isolation

- Absolute ethanol
- 75 % ethanol
- 8 mM NaOH
- 0.1 M sodium citrate in 10 % ethanol

For protein isolation

- Isopropanol
- 1 % sodium dodecyl sulfate (SDS)
- 0.3 M guanidine hydrochloride (GuHCl) in 95% ethanol
- Absolute ethanol

For particular samples

- Homogenization apparatus (for tissue and certain cells only)
- Red Blood Cell Lysis Buffer, Cat. No. 11 814 389 001 (for white blood cells only)
- Glycogen (for processing <10 mg tissue)

IV. Average nucleic acid yield from various sources

Sample	RNA yield	DNA yield
Tissue:		
Liver	6 – 10 µg/mg tissue	3 – 4 µg/mg tissue
Spleen	6 – 10 µg/mg tissue	not determined
Kidney	3 – 4 µg/mg tissue	3 – 4 µg/mg tissue
Skeletal muscle or brain	1.0 – 1.5 µg/mg tissue	2 – 3 µg/mg tissue
Placenta	1 – 4 µg/mg tissue	2 – 3 µg/mg tissue
Cultured cells:		
Epithelial cells	8 – 15 µg/10^6 cells	not determined
Fibroblasts	5 – 7 µg/10^6 cells	not determined
Human, mouse, or rat cells	not determined	5 – 7 µg/10^6 cells

V. Protocol for preparing RNA, DNA, and protein from animal tissue (based on the method of Chomczynski, 1993)

For a detailed, step-by-step procedure and for tips on handling different types of sample, see the package insert supplied with the reagent.

Extraction
Add 1 ml TriPure per 50 – 100 mg tissue
Homogenize sample in tissue homogenizer

↓

Incubate 5 min at 15 to 25°C to dissociate nucleoprotein complexes

↓

Add chloroform (0.2 ml per 1 ml TriPure)
Shake vigorously 15 s; Incubate 2 – 15 min at 15 to 25°C

↓

Centrifuge 12,000 x *g*, 15 min at 2 to 8°C

↓

3 Phases: Aqueous (containing RNA) colorless
Interphase (containing DNA) white
Organic (containing protein) red

↓

Isolation of RNA
Transfer aqueous phase to new tube

↓

Precipitate with isopropanol
(0.5 ml per 1 ml TriPure)
Mix by inversion
Incubate 5 – 10 min at 15 to 25°C

↓

Centrifuge 12,000 x *g*, 10 min at 2 to 8°C
Discard suppernatant

↓

Wash pellet 1 x with 75% EtOH (1 ml EtOH per 1 ml TriPure)

↓

Centrifuge 7500 x *g*, 5 min at 2 to 8°C
Discard supernatant

↓

Isolation of DNA/protein
from remaining interphase and organic phase

↓

Precipitate with ethanol (EtOH)
(0.3 ml 100% EtOH per 1 ml TriPure)
Mix by inversion
Incubate 2 – 3 min at 15 to 25°C

↓

Centrifuge 2000 x *g*, 5 min at 2 to 8°C
Separate pellet and phenol/EtOH supernatant

↓

Isolation of DNA
Pellet

↓

Wash pellet in 10% EtOH/0.1 M sodium citrate
(1 ml per 1 ml TriPure)
Incubate 30 min at 15 to 25°C with occasional mixing

↓

Isolation of protein
Phenol/EtOH supernatant

↓

Precipitate with isopropanol
(1.5 ml per 1 ml TriPure)
Mix by inversion
Incubate 10 min at 15 to 25°C

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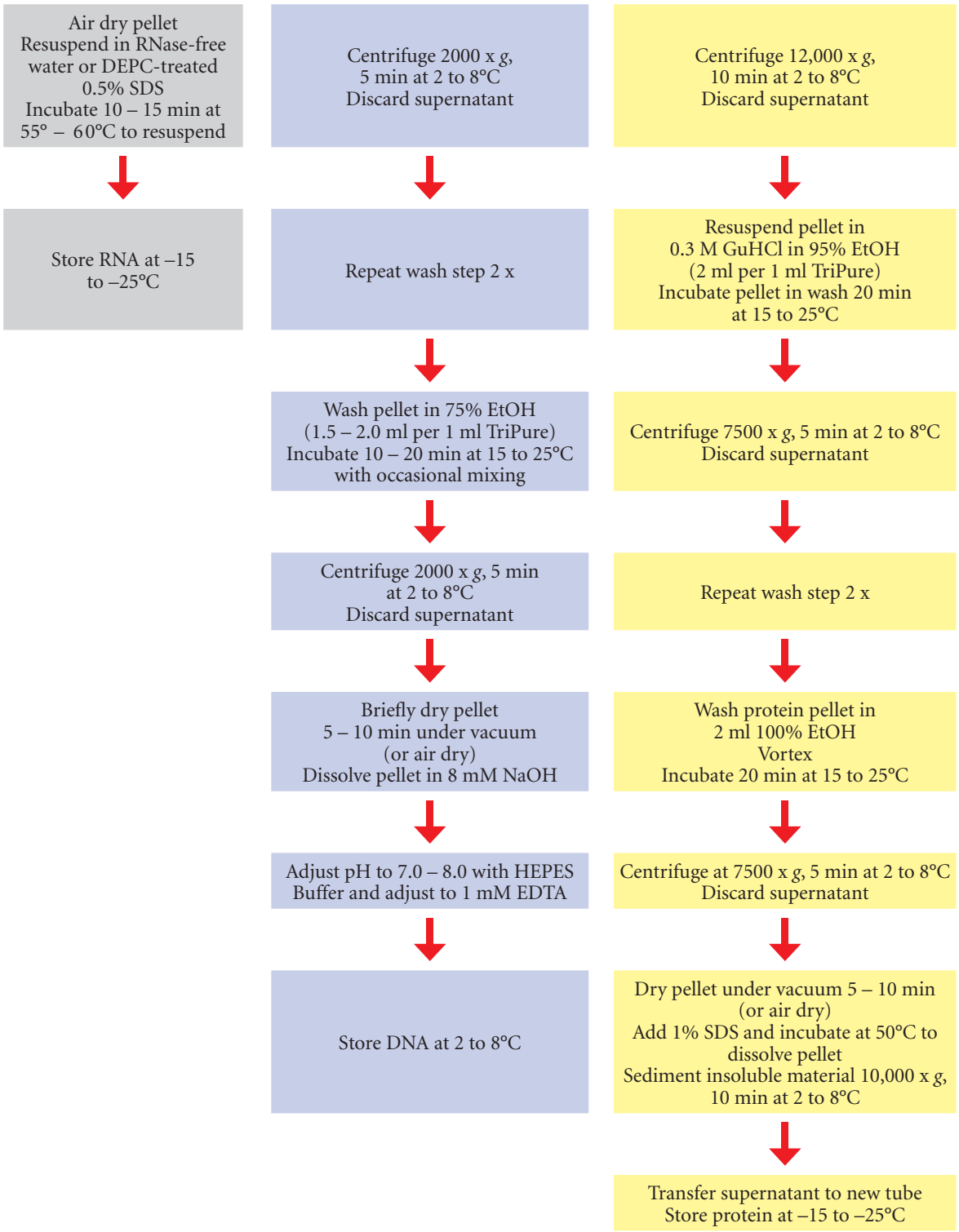
Air dry pellet
Resuspend in RNase-free water or DEPC-treated 0.5% SDS
Incubate 10 – 15 min at 55° – 60°C to resuspend
Store RNA at –15 to –25°C
Centrifuge 2000 x g, 5 min at 2 to 8°C
Discard supernatant
Repeat wash step 2 x
Wash pellet in 75% EtOH (1.5 – 2.0 ml per 1 ml TriPure)
Incubate 10 – 20 min at 15 to 25°C with occasional mixing
Centrifuge 2000 x g, 5 min at 2 to 8°C
Discard supernatant
Briefly dry pellet 5 – 10 min under vacuum (or air dry)
Dissolve pellet in 8 mM NaOH
Adjust pH to 7.0 – 8.0 with HEPES Buffer and adjust to 1 mM EDTA
Store DNA at 2 to 8°C
Centrifuge 12,000 x g, 10 min at 2 to 8°C
Discard supernatant
Resuspend pellet in 0.3 M GuHCl in 95% EtOH (2 ml per 1 ml TriPure)
Incubate pellet in wash 20 min at 15 to 25°C
Centrifuge 7500 x g, 5 min at 2 to 8°C
Discard supernatant
Repeat wash step 2 x
Wash protein pellet in 2 ml 100% EtOH
Vortex
Incubate 20 min at 15 to 25°C
Centrifuge at 7500 x g, 5 min at 2 to 8°C
Discard supernatant
Dry pellet under vacuum 5 – 10 min (or air dry)
Add 1% SDS and incubate at 50°C to dissolve pellet
Sediment insoluble material 10,000 x g, 10 min at 2 to 8°C
Transfer supernatant to new tube
Store protein at –15 to –25°C

V. Troubleshooting the TriPure Isolation protocol

During	If you get...	Then, the cause may be...	And you should...
RNA isolation	Low RNA yield	Incomplete homogenization or lysis of samples	▶ Use homogenizer to maximize sample yields.
		Incomplete solubilization of the final RNA pellet	▶ Do not let RNA pellet dry completely, as a dry pellet will be much less soluble. ▶ Increase incubation time to 30 min at 55°C to solubilize RNA.
	A_{260}/A_{280} ratio <1.65	Insufficient TriPure used for sample homogenization	▶ Add a sufficient volume of TriPure Isolation Reagent, according to package insert instructions.
		After homogenization, samples were not stored for 5 min at 15 to 25°C	▶ Store at 15 to 25°C for 5 min.
		Contamination of aqueous phase with phenol phase	▶ Carefully remove the upper aqueous phase for subsequent RNA iolation, making sure to avoid the interphase/organic phase.
		Incomplete solubilization of the final RNA pellet	▶ Increase incubation time to 30 min at 55°C to solubilize RNA.
	RNA degradation	Tissues were not immediately processed or frozen after removal from animal Samples used for isolation procedure were stored at –20°C instead of –70°C	▶ Use fresh tissue or tissue that has been directly frozen in liquid nitrogen and stored at –70°C prior to RNA isolation.
		Cells were dispersed by trypsin digestion	▶ Add TriPure Isolation Reagent directly to cells attached to culture dish or flask, according to package insert instructions.
		Aqueous solutions or tubes were not RNase-free	▶ Use sterile disposable plasticware and pipettes/tips reserved for RNA work only. ▶ Take appropriate precautions to ensure RNase-free environment.
	DNA contamination	Insufficient TriPure used for sample homogenization	▶ Add a sufficient volume of TriPure Isolation Reagent, according to package insert instructions.
		Starting samples contained organic solvents (EtOH, DMSO) or strong buffers; or had an alkaline pH	▶ Carefully remove the upper aqueous phase for subsequent RNA isolation, making sure to avoid the interphase/organic phase.

▶▶▶

V. Troubleshooting the TriPure Isolation protocol, continued

During	If you get...	Then, the cause may be...	And you should...
DNA isolation	Low DNA yield	Incomplete homogenization or lysis of samples	▶ Use power homogenizer to maximize sample yields.
		Incomplete solubilization of the final DNA pellet	▶ Do not let DNA pellet dry completely, as a dry pellet will be much less soluble.
	A_{260}/A_{280} ratio <1.7	Incomplete removal of phenol from the DNA preparation (during ethanol/sodium citrate wash)	▶ Incorporate an additional sodium citrate/ethanol wash step.
	DNA degradation	Tissues were not immediately processed or frozen after removal from animal Samples used for isolation procedure were stored at –20°C instead of –70°C	▶ Use fresh tissue or tissue that has been directly frozen in liquid nitrogen and stored at –70°C prior to DNA isolation.
		Samples were homogenized with a high speed homogenizer	▶ Avoid using power homogenizer. Use hand-held homogenizer to minimize shearing of high molecular weight DNA.
	RNA contamination	Too much aqueous phase remained with the interphase and organic phase	▶ Carefully remove all of the upper aqueous phase prior to isolation of DNA.
		Inadequate wash of DNA pellet with 10% EtOH/0.1 M sodium citrate	▶ After adding 1 ml sodium citrate/ethanol for each 1 ml TriPure Isolation Reagent (required in the initial homogenization process) incubate the sample, with occasional mixing, for 30 min at 15 to 25°C.
Protein isolation	Low protein yield	Incomplete homogenization or lysis of samples	▶ Use power homogenizer to maximize sample yields.
		Incomplete solubilization of the final protein pellet	▶ Incubate sample at 50°C to completely solubilize the protein.
	Protein degradation	Tissues were not immediately processed or frozen after removal from animal	▶ Use fresh tissue or tissue that has been directly frozen in liquid nitrogen and stored at –70°C prior to protein isolation.
	Deformed bands in PAGE analysis	Protein pellet not washed sufficiently	▶ Incorporate an additional wash step.

Typical result with the reagent

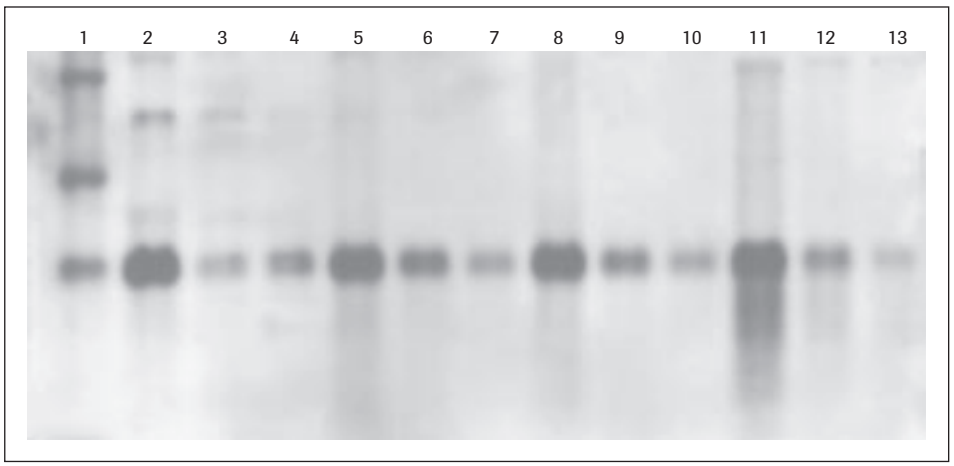

Figure 44: Northern blot with total RNA isolated by the TriPure Isolation Reagent. Total RNA was isolated (by the TriPure protocol) from the following research samples: 1.5 x 10^6 cells of a human leukemia cell line, 5.0 x 10^7 human white blood cells, 1.7 x 10^7 buffy coat cells from human blood, and 500 mg rat tissue. The isolated RNA samples were separated electrophoretically on a gel, transferred to a nylon membrane, and hybridized with a 1 kb, digoxigenin-labeled glyceraldehyde 3-phosphate dehydrogenase (G3PDH) probe. The blot was incubated overnight with DIG System reagents for chemiluminescent detection, then exposed to X-ray film for 5 min. The G3PDH probe recognizes a 1.35 kb mRNA, as shown in:

Lane 1: RNA ladder
Lanes 2 – 4: RNA from human leukemia cell line
Lanes 5 – 7: RNA from human white blood cell pellet
Lanes 8 – 10: RNA from human blood buffy coat
Lanes 11 – 13: RNA from rat liver tissue
The amount of total RNA applied to the original gel was either 5 µg (lanes 2, 5, 8, 11), 1 µg (lanes 3, 6, 9, 12), or 0.25 µg (lanes 4, 7, 10, 13).

Result: This data clearly demonstrates that high quality, intact RNA is successfully isolated from a variety of starting materials using the TriPure Isolation Reagent.

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