

Reagents

TRI Reagent® RNA Isolation Reagent

For isolation of total RNA from a variety of starting materials

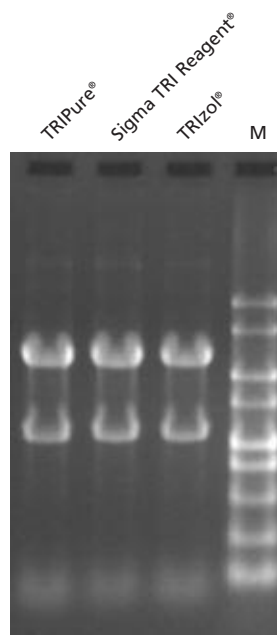
TRI Reagent® is an improved version of the single-step total RNA isolation reagent developed by Chomczynski.¹ The RNA isolation method based on this reagent is widely recognized and proven for RNA applications and is supported by a substantial publication list.² It is ideal for quick, economical, and efficient isolation of total RNA or the simultaneous isolation of RNA, DNA, and proteins from samples of human, animal, plant, yeast, bacterial and viral origin.

Features and Benefits

- Easily scalable RNA isolation
- Works with many sources: human, plant, yeast, bacterial, or viral
- Better yields than traditional guanidine thiocyanate/cesium chloride methods
- Three convenient formulations of TRI Reagent®

Storage: 2-8 °C

R: 23/24/25-32-34-48/20/21/22-54-53-68 S: 26-36/37/39-45-61



Total RNA was prepared from HeLa cells using TRI Reagent® from Sigma and equivalent reagents from other various suppliers

Figure 1. Total RNA from HeLa cells was prepared using TRI Pure®, Sigma TRI Reagent®, and TRIzol®. An aliquot of total RNA was analyzed on a 1% agarose gel. RNA Marker (M) ranged from 0.2 bp-10 kb (Cat. No. R7020).

Table 1. TRI Reagent® Formulations

Product Name	Sample Type	Sample Volume	TRI Reagent® Volume
TRI Reagent®	Tissues, cultured adherent cells, cell pellets	up to 100 mg tissue, 10 ⁷ cells, or 10 ² cm plate area	1 ml
TRI Reagent® BD	Whole blood, plasma, serum	0.25 ml blood derivatives	0.75 ml
TRI Reagent® LS	Cell suspension, CSF, amniotic fluid	0.25 ml fluid samples	0.75 ml

Table 2. Typical RNA Yield

Tissue	Yield (µg RNA/mg tissue)
Liver	6-10
Spleen	6-10
Kidney	3-4
Skeletal Muscle	1-1.5
Brain	1-1.5
Placenta	1-4

Cell	Yield (µg RNA/10 ⁶ cells)
Epithelial	8-15
Fibroblast	5-7

Ordering Information

Cat. No.	Product Description	Quantity
T9424	TRI Reagent® RNA, DNA and Protein Isolation Reagent	25 ml 100 ml 200 ml 6 x 100 ml
T3809	TRI Reagent® BD	25 ml 100 ml 200 ml
T3934	TRI Reagent® LS	25 ml 100 ml 200 ml

References

1. Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156 (1987).
2. Chomczynski, P. and Mackey, K., Short Technical Reports. Modification of the TRI Reagent® procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *BioTechniques* **19**: 924-945 (1995).

Reagents

Molecular Biology Reagent Ethanol

Sigma offers both 100% and 95% non-denatured Molecular Biology Reagent Ethanol. Both products are application tested to verify DNA and RNA precipitation and the absence of nucleases. Both are offered as Excise Tax included. These products are benzene-free, and meet ACS requirements. Some sales restrictions may apply outside of the United States.
[64-17-5] CH₃CH₂OH

Cat. No.	Product Description	Quantity
E7023	Ethanol, Absolute, 100% (200 proof) (Ethyl Alcohol) Water ≤ 0.05% Molecular Biology Reagent, DNase, and RNase Free Excise Tax included: No ATF license required Suitable for use in the precipitation of nucleic acids.	500 ml 6 x 500 ml 4 x 4 L
E7148	Ethanol, Absolute, 95% (190 proof) (Ethyl Alcohol) 95+% Ethanol Molecular Biology Reagent, DNase, and RNase Free Excise Tax included: No ATF license required Suitable for use in the precipitation of nucleic acids.	1 gal 4 x 1 gal 500 ml 6 x 500 ml

GenElute™ LPA Linear Polyacrylamide

GenElute LPA Linear Polyacrylamide is an efficient neutral carrier for **precipitating picogram amounts of nucleic acids with ethanol**. The nucleic acid precipitate can be collected simply by centrifugation. LPA offers several advantages for recovering DNA or studying DNA-protein interactions, relative to other carriers, such as tRNA or glycogen. tRNA interferes with DNA during phosphorylation with polynucleotide kinase and glycogen competes with protein in DNA-protein interaction studies. In contrast, LPA is completely inert. LPA is synthesized chemically and, therefore, is not contaminated with biological material. The precipitate is visible immediately upon addition of LPA, thus eliminating wait time and low temperature incubation.

The presence of LPA during ethanol precipitation results in complete recovery of fragments larger than 20 base pairs, whereas most of the DNA is lost if no carrier is used. Very short DNA fragments, less than 20 base pairs, do not co-precipitate with LPA¹, allowing separation of labeled DNA from unreacted nucleotides by precipitation after the labeling reaction.

LPA has been used in several laboratories for most of the common manipulations of DNA, including automated sequencing, enzyme reactions, gel electrophoresis, cloning², and DNA-protein interactions^{1,3}, and appears inert in all experiments. A very small amount of LPA is required as carrier during ethanol precipitation of DNA.

GenElute-LPA is tested for nucleases, and is supplied in nuclease-free water as a 5-mg/ml solution.

References

1. Gaillard, C. and F. Strauss, *Nucl. Acid Res.* **18**: 378 (1990).
2. Strauss, F. and A. Varshavsky, *Cell* **37**: 889-901 (1984).
3. Aruffo, A. and B. Seed, *Proc. Natl. Acad. Sci. USA* **84**: 8573-8577 (1987).

Cat. No.	Product Description	Quantity
56575	GenElute-LPA 5 mg/ml in nuclease-free water	5 x 1 ml



Reagents

Glycogen, Molecular Biology Reagent

From mussels

RNase, DNase, Nickase, Protease, Nucleic Acids – None detected.
Each vial contains an aqueous solution of Glycogen (approximately 20 mg in 1 ml). It is intended as a carrier molecule for DNAs and RNAs, replacing tRNAs or sonicated DNAs.

Cat. No.	Product Description	Quantity
G1767	Glycogen, Molecular Biology Reagent	1 vial

Guanidine Hydrochloride

Forms a clear, colorless solution at 6 M

Pb \leq 5 ppm

DNase and RNase: None detected.

[50-01-1] $\text{CH}_5\text{N}_3 \cdot \text{HCl}$ FW 95.53

Cat. No.	Product Description	Quantity
G3272	Guanidine Hydrochloride (Aminomethanamide) Hydrochloride	25 g 100 g 500 g 1 kg 2 kg

Guanidine Hydrochloride Solution

8 M in H_2O (filtered)

d = 1.186 g/ml

[50-01-1]

Cat. No.	Product Description	Quantity
G9284	Guanidine Hydrochloride Solution	100 ml 500 ml

Guanidine Thiocyanate

Assay: \geq 99%

DNase and RNase: None detected.

[593-84-0] $\text{CH}_5\text{N}_3 \cdot \text{HSCN}$ FW 118.2

Cat. No.	Product Description	Quantity
G9277	Guanidine Thiocyanate	100 g 250 g 500 g 6 x 500 g

Isopropanol

Suitable for use in the precipitation of nucleic acids

When compared to ethanol, 50% less is required for nucleic acid precipitation, thus minimizing the total volume to be centrifuged for DNA or RNA recovery.

Water: \leq 0.05%

[67-63-0] $\text{C}_3\text{H}_8\text{O}$ FW 60.10

Reference

1. Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989) p. E.13-E.14.

Cat. No.	Product Description	Quantity
I9516	Isopropanol (2-Propanol) Purity: 99+%	25 ml 4 x 25 ml 500 ml

Reagents

Lysozyme

Hydrolyzes the β -1,4 linkages between N-acetylmuramic acid and N-acetylglucosamine in the cell wall structure of many microorganisms. This is particularly useful for lysing gram positive and gram negative bacteria for subsequent nucleic acid extraction. Each lot is use tested in the isolation of plasmid DNA from *E. coli*.

Approximately 95% protein; balance primarily buffer salts as sodium acetate and sodium chloride.

Activity: Approximately 50,000 units per mg protein ($E_{282}^{1\%}$).

Unit Definition: One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 ml reaction mixture (1 cm light path).
M.W. approximately 14,300.
[12650-88-3, EC 3.2.1.17]

References

1. Jolles, P., *Angew. Chem. Int. Ed.*, **8**: 227 (1969).
2. Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory p. 1.29 (1989).

Cat. No.	Product Description	Quantity
L7651	Lysozyme (Muramidase; mucopolysaccharidase) N-acetylmuramoylhydrolase) From Chicken Egg White 3x Crystallized, dialyzed and lyophilized	1 g 5 g 10 g 25 g 100 g

Polyethylene Glycol

Av. Mol. Wt.: 8000
DNase, RNase: None detected.
[25322-68-3]

Cat. No.	Product Description	Quantity
P5413	Polyethylene Glycol	500 g 1 kg 2 kg

Proteinase K

From *Tritirachium album*

(EC 3.4.21.64)
No detectable endonuclease (nickase), endonuclease-exonuclease or RNase activity. Specific conditions given on accompanying data sheet. <0.5 ppm DNA using PicoGreen® assay
[39450-01-6]

Unit Definition: One unit will hydrolyze casein to produce color equivalent to 1.0 μ mole (181 mg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

Activity: 10-20 units per mg protein.

Cat. No.	Product Description	Quantity
P2308	Proteinase K Lyophilized powder containing minimum 90% protein (Biuret)	5 mg 10 mg 25 mg 100 mg 500 mg 1 g

Proteinase K Solution

From *Tritirachium album*

(EC 3.4.21.64)
No detectable endonuclease (nickase), endonuclease-exonuclease or RNase activity. <0.5 ppm DNA using PicoGreen® assay
[39450-01-6]

Solution in 40% glycerol (v/v) containing 10 mM Tris-HCl, pH 7.5, with 1 mM calcium acetate.

Prepared from P2308

Unit Definition: One unit will hydrolyze urea-denatured hemoglobin to produce color equivalent to 1.0 μ mole (181 mg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

Cat. No.	Product Description	Quantity
P4850	Proteinase K Solution Minimum 140 units/ml, Buffered aqueous glycerol solution	5 ml

Reagents

Ribonuclease A

From Bovine pancreas

Ribonuclease A (RNase A) is commonly used to degrade RNA in DNA preparations.
(EC 3.1.27.5)
[9001-99-4]

Activity: ≥ 70 Kunitz units per mg protein

Functional Assay: Tested in plasmid purification

Detection Limit: Degradation of 10% of the DNA

At concentrations up to 25 mg per ml, nicking or degradation of plasmid is not detectable.

Reference

1. Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 451 (1983).

Cat. No.	Product Description	Quantity
R6513	Chromatographically purified,	10 mg
	lyophilized powder	50 mg
	Note: Boiling of stock solutions to	250 mg
	inactivate residual DNase is not	500 mg
	necessary or recommended.	1 g
R4642	Solution in 50% glycerol containing	10 mg
	10 mM Tris-HCl, pH 8.0	50 mg
	Note: Boiling of solutions to	250 mg
	inactivate DNase is unnecessary	1 g
	and is not recommended.	

Ribonuclease Inhibitor

Source: Human Placenta

Solution in 50% glycerol, 20 mM HEPES-KOH, pH7.6, 50 mM KCl and 8 mM DTT

Useful for *in vitro* inhibition of ribonucleases, including procedures like cDNA synthesis, RT-PCR, and *in vitro* transcription and translation

Activity: Approximately 30,000-50,000 units per ml

Unit Definition: One unit will reduce the activity of 5 ng of ribonuclease A by 50% in a cytidine 2':3'-cyclic monophosphate system

Approximately 50 kDa

Storage: -20°C

Shipped in dry ice

Reference

1. Blackburn, P., Ribonuclease inhibitor from human placenta: interaction with derivatives of ribonuclease A. *J. Biol. Chem.*, **254**: 12488-12493 (1979).

Cat. No.	Product Description	Quantity
R2520	Ribonuclease Inhibitor	2,500 units
		10,000 units
		20,000 units

protectRNA™ RNase Inhibitor, 500x

A potent inhibitor of most nucleic acid binding enzymes, and thus useful as an RNase inhibitor. Especially useful when performing *in situ* hybridization. If it is added to all aqueous solutions used, it eliminates the need for special glassware washing and after-wash treatments. The 500x concentrate is economical; 2 ml treats 1,000 ml of solution. Not recommended in systems where other enzymatic activity is required.

Cat. No.	Product Description	Quantity
R7397	protectRNA™ RNase Inhibitor, 500x	30 ml

RNaseZAP®

A cleaning agent for removing RNase from glassware, plastic surfaces, countertops, and pipettors. It is also effective at eliminating RNase contamination from microcentrifuge tubes without inhibiting subsequent enzymatic reactions.

Cat. No.	Product Description	Quantity
R2020	RNaseZAP®	250 ml
		6 x 250 ml