CYRN02-CHANGYU TRIpure LS (Liquid Sample RNA Extraction Reagent)

Catalog number	Package
CYRN0201	50 ml
CYRN0202	100 ml

Storage: stable at 15-25 °C for 12 months. For best results store at 2-8 °C.

Description

TRIpure LS is a single-step reagent for the isolation of total RNA from liquid and solid samples of human, animal, plant, yeast, bacterial and viral origin. Cells are lysed and dissolved while RNA integrity is maintained. After addition of chloroform and centrifugation the sample separates into an aqueous phase and an organic phase; RNA remains in the aqueous phase and is recovered by isopropanol precipitation. After the aqueous phase is removed, DNA and protein can be recovered sequentially by precipitation.

The reagent is suitable for small (50-100 mg tissue, 5×10^6 cells) and large (1 g or more of tissue, more than 10^7 cells) samples, and allows several samples to be processed in parallel within one hour. Typical applications include Northern blotting, dot blot hybridisation, poly(A)+ selection, in vitro translation, RNase protection assays and molecular cloning. For PCR with primers located in a single exon, treatment of the RNA with amplification-grade DNase I is recommended. A260/A280 of RNA diluted in TE is 1.8 or higher.

Notes before starting

Note: TRIpure LS is toxic and can cause burns on contact with skin or if swallowed. Wash immediately with plenty of detergent and water and seek medical advice.

Note: Wear gloves and eye protection; avoid contact with skin and clothing; work in a chemical fume hood and avoid inhalation.

Note: Unless otherwise stated, carry out all steps at 15-30 °C.

Note: Use clean disposable polypropylene tubes when less than 2 ml of TRIpure LS is used. For larger volumes use glass (Corex) or polypropylene tubes after confirming that they withstand 12,000×g with TRIpure LS and chloroform. Never use cracked or broken tubes. Balance tubes and seal glass tubes with aluminium foil and parafilm.

Note: For 1-10 mg tissue or 10^2-10^4 cells, adjust the sample volume to 0.25 ml and add 0.75 ml TRIpure LS. Add 5-10 µg RNase-free glycogen as a carrier before isopropanol precipitation and pass the lysate twice through a 26-gauge needle before adding chloroform to reduce viscosity. Glycogen co-precipitates with RNA and, below 4 mg/ml, does not inhibit first-strand synthesis or PCR.

Note: After homogenisation and before adding chloroform, samples can be stored at -60 to -70 °C for at least one month. The RNA pellet in 75% ethanol can be stored at 2-8 °C for at least one week or at -5 to -20 °C for at least one year.

Note: If the centrifuge reaches only 2,600×g, extend the centrifugation in steps 2 and 3 to 30-60 minutes.

Reagents not supplied

Chloroform; isopropanol; 75% ethanol prepared with DEPC-treated water; RNase-free water or 0.5% SDS.

❖ Procedure

1. Homogenisation

a. Biological fluids: add 0.75 ml TRIpure LS to 0.25 ml liquid sample (serum, plasma, cerebrospinal fluid and similar) and pipette several times to lyse the cells. Use at least 0.75 ml TRIpure LS per 5-10×10^6 cells. The final ratio of TRIpure LS to sample is always 3:1.

b. Tissue: homogenise 50-100 mg tissue, or 0.25 ml tissue suspension, in 0.75 ml TRIpure LS with a glass or high-power homogeniser. If the tissue volume is below 0.25 ml, add sterile water to 0.25 ml to keep the 3:1 ratio.

c. Adherent cells: add 0.3-0.4 ml TRIpure LS directly to a 3.5 cm dish and pipette to lyse. Base the volume on the dish area, not on cell number (0.3-0.4 ml per 10 cm^2). No water is needed because residual medium already dilutes the reagent.

d. Suspension cells: pellet by centrifugation and lyse by pipetting in TRIpure LS. Use 0.75 ml per 5-10×10^6 animal, plant or yeast cells or per 1×10^7 bacteria, and adjust the sample volume to 0.25 ml with sterile water. Do not wash cells before adding the reagent. Some yeast and bacteria require homogenisation.

Optional: for samples rich in protein, fat, polysaccharide or extracellular material, centrifuge the homogenate at 12,000×g for 10 minutes at 2-8 °C and transfer the clear homogenate to a clean tube. Remove the fat layer from adipose samples.

2. Phase separation

Incubate the homogenate at 15-30 °C for 5 minutes. Add 0.2 ml chloroform per 0.75 ml TRIpure LS, cap tightly, shake by hand for 15 seconds and incubate at 30 °C for 2-15 minutes. Centrifuge at no more than 12,000×g for 15 minutes at 2-8 °C. The mixture separates into a lower phenol-chloroform phase, an interphase and an upper colourless aqueous phase; RNA is in the aqueous phase, which is about 70% of the TRIpure LS volume.

3. RNA precipitation

Transfer the aqueous phase to a clean tube; keep the organic phase if DNA and protein are required. Add 0.5 ml isopropanol per 0.75 ml TRIpure LS used for homogenisation, incubate at 15-30 °C for 10 minutes and centrifuge at no more than 12,000×g for 10 minutes at 2-8 °C. The RNA pellet is usually invisible before centrifugation and forms a gel-like film on the tube wall and bottom.

4. RNA wash

Remove the supernatant and wash the pellet once with 75% ethanol, using at least 1 ml per 0.75 ml TRIpure LS. Vortex and centrifuge at no more than 7,500×g for 5 minutes at 2-8 °C.

5. RNA dissolution

Air-dry or vacuum-dry the pellet for 5-10 minutes; do not centrifuge-dry. Do not let the pellet dry completely. Dissolve the RNA in RNase-free water or 0.5% SDS (avoid SDS if the RNA will be used in enzymatic reactions) and incubate at 55-60 °C for 10 minutes. RNA can also be dissolved in 100% deionised formamide and stored at -70 °C.

Typical yield

Sample	Expected RNA yield
Whole blood (human or animal)	15-20 μg per ml
Human lymphocytes (about 7×10^7 leukocytes)	60-70 μg
Liver and spleen	6-10 μg per mg tissue
Kidney	3-4 μg per mg tissue
Skeletal muscle and brain	1-1.5 μg per mg tissue

Placenta	1-4 μg per mg tissue
Epithelial cells (1×10^6 cultured cells)	8-15 μg
Fibroblasts (1×10^6 cultured cells)	5-7 μg

Troubleshooting

Problem	Possible cause and action
Low yield	Incomplete homogenisation or lysis; incomplete dissolution of the final pellet.
A260/A280 below 1.65	RNA diluted in water rather than TE; too little reagent; sample not held at room temperature for 5 minutes after homogenisation; aqueous phase contaminated with phenol; RNA not fully dissolved.
RNA degradation	Tissue not processed or frozen immediately; samples stored at -5 to -20 °C instead of -60 to -70 °C; trypsin-dispersed cells; RNase-contaminated solutions or tubes.
DNA contamination	Too little reagent during homogenisation; sample contained organic solvents, strong buffers or alkaline solutions.
Polysaccharide and proteoglycan contamination	Add 0.25 ml isopropanol and then 0.25 ml high-salt solution (0.8 M sodium citrate, 1.2 M NaCl) per 1 ml TRIpure LS in the aqueous phase and continue.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.