

# peqGOLD RNAPure™ System

For in vitro research use only

## Lot-No.

Order No.	30-1010	100ml
	30-1020	200ml
	30-1030	500ml

Shipment	At ambient temperature
Preparation	Ready to use
Storage	Dark, at 4° C
Stability	Stable for 12 month

## Introduction

The isolation of pure and undegraded RNA from a large number of samples in a short time is a prerequisite for many experiments in molecular biology. Chirgwin et al. have shown (1) that using guanidinium thiocyanate and LiCl centrifugation yields good RNA from many tissues. This method turned out to be unsatisfying with tissues or cells containing high amounts of RNase. Recent progress i.e. inclusion of phenol and chloroform has made it possible to obtain excellent RNA preparations within 1-2 hours free of DNA in a single-step procedure. This method provides very high yield and purity of RNA preparations.

The ease of the **peqGOLD RNAPure™** method combined with excellent recoveries from small quantities of tissue or cells makes our product especially suitable for gene expression studies. RNA preparations are essentially undegraded, free of DNA and proteins and contain the entire spectrum of RNA molecules, including small (4-5 S) RNAs. The RNA is ready for Northern blotting experiments, dot blot hybridization, and polyA<sup>+</sup> selection for mRNA isolation. It can be directly used for molecular cloning, in vitro translation and RNase protection experiments.

## Warning

peqGOLD RNAPure Isolation Reagent contains phenol (a poison) and guanidine thiocyanate (an irritant). This reagent is toxic and **CAN BE FATAL** if it is swallowed. This reagent can also cause burns if it touches the skin.

When using peqGOLD RNAPure Isolation Reagent, work under a fume hood. Wear gloves, lab coat, and appropriate eye protection (shield, safety goggles). Avoid breathing vapor.

**IF YOU GET THE REAGENT ON YOUR SKIN**, wash skin immediately with soap or mild detergent, and flush with large amounts of water for 15–30 minutes, until no evidence of chemical remains. Seek medical attention.

**IF YOU GET THE REAGENT IN YOUR EYES**, flush eyes with large amounts of water for 15–30 minutes, until no evidence of chemical remains. Seek medical attention.

## peqGOLD RNAPure™ Method

### Reagents required, but not supplied

Chloroform p.A.  
Isopropanol p.A.  
70% Ethanol p.A.

RNA isolation by the peqGOLD RNAPure™ method includes the following steps:

- |                      |   |
|----------------------|---|
| 1. Homogenization    | peqGOLD RNAPure™ (2 ml/100 mg tissue or $10^7$ cells) |
| 2. RNA Extraction    | 1 vol. homogenate + 0.1 vol. Chloroform               |
| 3. RNA Precipitation | 1 vol. isopropanol                                    |
| 4. RNA Wash          | 70% ethanol   |

### Homogenization

- Homogenize tissue samples with peqGOLD RNAPure™ (2 ml per 100 mg tissue) with a few strokes in a glass-Teflon homogenizer.
- To isolate RNA from cells grown in suspension, sediment cells and lyse them by the addition of 1 ml peqGOLD RNAPure™ per  $5-10 \times 10^6$  cells.  
Cells grown in monolayer are lysed directly in the culture dish by the addition of peqGOLD RNAPure™ (1 ml per 3.5 cm petri dish). Solubilize RNA by passing the lysate a few times through the pipette.

### RNA Extraction

Incubate each homogenized sample for 5 minutes at +15 to +25°C to ensure the complete dissociation of nucleoprotein complexes.

Add chloroform to each sample. Use 0.2 ml chloroform for each 1 ml RNAPure Isolation Reagent required in the initial homogenization. Cap tube securely, and shake it vigorously for 15 seconds. Incubate tube on ice (or at 4°C) for 5 minutes.

To separate the solution into three phases, centrifuge tube at 12,000 x *g* for 15 minutes at 4°C.

After centrifugation, use the colorless upper aqueous phase for the RNA isolation. The lower organic phase and the interphase contain the DNA and proteins.

The volume of the aqueous phase is about 50% of the initial volume of peqGOLD RNAPure™ plus the volume of tissue used for homogenization.

### RNA Precipitation

Transfer the aqueous phase to a fresh tube, add an equal volume of isopropanol, mix and store the samples for 15 minutes at 4°C. Centrifuge samples for 15 minutes at 12,000 g (4°C). RNA precipitate (often invisible before centrifugation) forms a white-yellow pellet at the bottom of the tube.

## RNA Wash

Remove the supernatant and wash the RNA pellet once with 75% ethanol by vortexing and subsequent centrifugation for 10 minutes at 12,000 x g (4°C). Repeat the washing step if necessary (phenol smell!). Use at least 0.8 ml of ethanol per 50-100 µg RNA.

Remove the excess ethanol from the RNA pellet by air-drying or placing the sample under vacuum for 5–10 minutes.

*Caution: Do not dry the RNA pellet by centrifugation under vacuum. Do not let the RNA pellet dry completely as a dry pellet will be much less soluble.*

Resuspend the RNA pellet in DEPC-treated 0.5% SDS, in 1 mM EDTA, pH 7 or RNase-free water. Dissolve the RNA pellet by passing the solution through a pipette tip several times, then incubating the solution for 10 -15 minutes at 55°C to 60°C.

The final preparation is free of DNA and proteins and has usually a 260/280 ratio between 1.6 and 2.0. If the final RNA preparation still contains UV-absorbing material (see introduction) that interferes with the following experiments continue with the method described in the next chapter.

## Notes and Comments

- To avoid RNase contamination use gloves and sterile disposable polypropylene tubes and keep the tubes closed during the entire procedure.
- If small RNA yields are expected (<10µg) use 70 µg Glycogen per 1 ml peqGOLD RNAPure™ as a carrier for the precipitation.
- After homogenization i.e. before the addition of chloroform samples can be stored at -70°C for a few months. The RNA precipitate from step 4 can be stored in 75% ethanol for 1-3 weeks at 4°C or for at least one year at -20°C.
- For samples with high content of proteins, polysaccharides, fat or other materials an extra purification step should be included.

After homogenization do not proceed to phase separation with chloroform but rather remove insoluble material by centrifugation at 12.000 g for 10 minutes at 4°C. The supernatant contains RNA while the pellet consists of polysaccharides, extra cellular membranes and high molecular weight DNA. Samples from fat tissues will result in a floating fat layer that should be removed. Transfer the clear RNA supernatant to a fresh tube and proceed with the phase separation as described.

## Trouble shooting Guide RNA isolation

Expected yields of RNA per mg of tissue or 10<sup>6</sup> cells

Liver and spleen	6-10 µg
Kidney	3-4 µg
Skeletal muscles and brain	1-1.5 µg
Placenta	1-4 µg
Epithelial cells	8-15 µg
Fibroblasts	5-7 µg

## peqGOLD RNAPure™ - Protocol

### Low yield

- Incomplete homogenization or incomplete lysis
- Final RNA pellet not completely dissolved

### $A_{260}/A_{280}$ ratio < 1.65

- Sample homogenized in too small volume
- After homogenization samples were not stored at room temperature for 5 minutes
- The aqueous phase was contaminated with the phenol phase
- Incomplete dissolution of the final RNA pellet

### RNA degradation

- Tissues were not immediately processed or frozen
- Cells were dispersed by Trypsin
- RNase contamination during preparation
- Samples used for isolation were frozen at  $-20^{\circ}\text{C}$  instead of  $-70^{\circ}\text{C}$
- Formaldehyde solution used for electrophoresis had a pH below 3.5

### DNA contamination

- Samples homogenized in too small volume
- Samples used for the isolation contained organic solvents (ethanol, DMSO, strong buffers or alkaline pH)

## **References**

1. J.M. Chirgwin, A.E. Przybyla, R.J. MacDonald and W.J. Rutter, *Biochemistry*, 18, 5294 - 5299 (1979)
2. E.I. Devinoy, C. Hubert, G. Jolivet, D. Thepot, N. Clergue, M. Desaleux, M. Dion, J.L. Serveley and L.M. Houdebine, *Reprod. Nutr. Develop.*, 28, 1145 - 1164 (1989)
3. T. Maniatis, E.F. Fritsch and J. Sambrook, *Molecular Cloning*, Cold Spring Harbor Laboratory, 188 - 209 (1982)