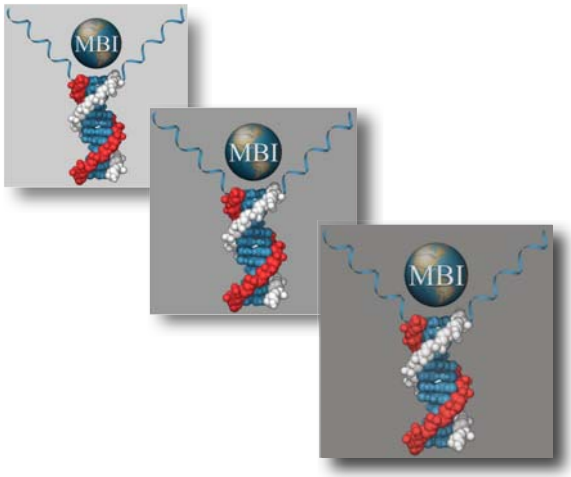
GStract™ RNA Isolation Kit II
Guanidinium Thiocyanate Method

Catalog No.
SA-40006: 50 preparation
SA-40005: 100 preparation



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Introduction
Isolation of high-quality, full-length RNA from tissue is fundamental importance to the practice biology. Successful techniques must be capable of inhibiting ubiquitous RNase until the RNA is separated from cellular proteins. Guanidinium thiocyanate is one of the most effective chaotropic reagents and is widely used for RNA isolation as it is a strong inhibitor of ribonuclease. The Total RNA Isolation Kit is based on the procedure of Chomczynski and Sacchi. The procedure has been modified to include further extraction step to eliminate DNA, polysaccharide and other contaminates that can be a problem with guanidine-based methods. The product of RNA is ready for Northern blot analysis, RNase protection assays, RT-PCR, Poly(A)$^+$ selection and in vitro translation.

Storage
All components of this kit are stable at least for six to nine months when stored at 4°C.

Reagents Supplied

Reagent/Cat. No.	SA-40006	SA-40005
GS1	50 ml	100 ml
Salt Solution	10 ml	20 ml
GS2	50 ml	100 ml
DEPC-H_2O	2 x 1.5 ml	4 x 1.5 ml

Reagents Needed:
H_2O-saturated phenol
Chloroform (ACS grade)
Isopropanol (ACS grade)
75% Ethanol (ACS grade)

Basic Protocol:
- Homogenize tissue/cells with **GS1**
- Add **Salt Solution** to precipitate the DNA
- Extract the RNA with phenol-chloroform
- Precipitate the RNA with Isopropanol
- Wash the RNA pellet with **GS2** to eliminate all contaminants.

Procedure:
Note: All centrifugation should be performed at 10,000 to 12,000 xg. Microcentrifugation should be performed at the maximum speed.

Part I - Sample Preparation:

A. ***Tissue***: Homogenize tissue sample in **GS1** (1 ml per 50-100 mg tissue) in a homogenizer.

B. ***Cells grown in suspension***:
Cells are sedimented at 1,000 xg for 5-10 minutes and the cell pellets are lysed in the **GS1** (1 ml per 5-10 X 10^6 cells).

C. *Cells grown in monolayer*:
Cells are lysed directly in the culture dish in the **GS1** (1 ml per 100 mm culture dish).

D. *Bacteria*:
Spin down the overnight bacterial culture at 5,000 xg for 5 minutes and the cell pellets are lysed in GS1 (1 ml per 1-5 ml of bacterial culture).

Part II. General Procedure Followed by Sample Preparation:

1. Completely lyse the cells by passing the lysate a few times through the pipette.
2. Add 200 µl of **Salt Solution** and mix well by vortexing for 10 seconds.
3. Add 1 ml of H_2O-saturated phenol and 300 µl of chloroform, mix by vortexing for 10 seconds and place on ice for 5 minutes.
4. Centrifuge the mixture for 10 minutes at 4 °C.
5. Transfer the aqueous phase (upper phase) to a fresh tube, add 1 ml **ice-cold** isopropanol.
6. Centrifuge RNA samples for 15 minutes at 4 °C. RNA precipitate (often invisible before centrifugation) forms a white pellet at the bottom of the tube.
7. Remove the supernatant and agitated the RNA pellet with 1 ml **GS2** by vortexing 5-10 seconds. (See Note)
8. Centrifuge for 5 minutes and discard the supernatant.
9. Wash the RNA pellet with cold 70% ethanol, centrifuge for 5 minutes and discard the supernatant. (See Note)
10. Dry briefly the RNA pellet under vacuum for 5-10 minutes (It is important not to let the RNA pellet dry completely, as it will greatly decrease its solubility).
11. Dissolve the RNA pellet with DEPC-treated H_2O. (See Note)

Notes:

- The procedure of steps 7-8 helps to remove all the carbonhydrate and polysaccharides from the RNA pellet. User can skip these steps and proceed directly from step 6 to step 9.
- To help dissolving the RNA, after adding the DEPC- H_2O, it can be put at 65°C for minutes and ice-quench.

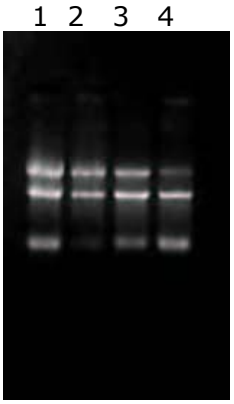

Fig 1. Total RNA was isolated from different sources.
Lane 1: Mouse Brain Tissue
Lane 2: Bovine Lung Tissue
Lane 3: Chinese Hamster Ovary (CHO) Cells
Lane 4: BeWo Cells

References:

1. Chomczynski, P. et. al. (1987). *Anal. Biochem*. **162**: 156-159
2. Farrell, R.E., Jr *RNA Methodologies*: A Laboratory Guide for Isolation and Characterization. Chapter 5.
3. Grope J. C. et. al. (1993) Anal. *Biochem*. **210**: 337-343.
4. Macfarlane D.E. et. al. (1993) *Nature* **362**: 186-188.
5. Maniatis, T., E.F. Fritsch and J. Sambrook. (1989). *Molecular Cloning*: A Laboratory Press, Cold Spring Harbor, NY.