

## Total RNA Isolation Reagent 10603

For research use only

### INTRODUCTION

The Total RNA Isolation Reagent is dedicated for the isolation of total RNA from cells cultures or animal tissues. It's a simple alternative derived from Chomczynski technique\*.

\* P. Chomczynski and N. Sacchi (1987), Anal. Biochem. **162**, 156-159.

### PRODUCT DESCRIPTION

The Total RNA Isolation Reagent is a ready to use solution allowing performing purification from a wide range of cells and tissues. The Total RNA purified is directly usable for RT-PCR, Northern Blot, *in vitro* translation or mRNA purification (see related products).

Typical yield: 10-15µg / 10<sup>6</sup> cells  
Purity: A<sub>260/280</sub> = 1.7 - 1.9 without DNA and proteins contamination.

### INSTRUCTIONS FOR USE

#### A. RNA Extraction from cells

**From Cells in suspension:** After cells counting and centrifugation, add 2ml of Total RNA Isolation Reagent per 10<sup>7</sup> cells.

**From Cells grown as a monolayer:** add directly 1ml of Total RNA Isolation Reagent per 3.5cm Ø dish. Then repeat pipetting to complete the lyse of the cells.

1. Add 0.2ml chloroform for 2ml of homogenate and then shake vigorously during 15-30 seconds. Incubate on ice for 5 min.
2. Centrifuge the mixture at 12000 x g for 15 min at 4°C. The mixture should present 2 phases. The colourless upper aqueous phase (containing the RNA), the blue lower organic phase and the interphase (containing DNA and proteins).
3. Transfer the aqueous phase (colourless upper phase) into a clean tube and add 1 volume of isopropanol. Then incubate at room temperature for 5-10 minutes.
4. Centrifuge at 12000 x g for 5 min at 4-25°C.
5. Remove the supernatant and wash the RNA pellet with ethanol solution 75% (use at least 1ml of ethanol solution per ml of Total RNA Isolation Reagent). Mix well and centrifuge at 7500 x g for 5 min at 4-25°C. A second round could be necessary to improve the A<sub>260/280</sub> ratio.
6. Air dry the pellet. Do not dry under vacuum centrifugation.
7. Resuspend the RNA in an appropriate volume of RNase Free Water by pipetting. If necessary heat to about 55°C during 5-10 minutes if necessary.

#### B. RNA Extraction from tissue

Efficient disruption and homogenization of the starting material is important. Complete disruption is absolutely required to release all the RNA contained in the sample. Homogenization reduces the viscosity of the lysates produced by disruption. Appropriate mechanical homogenizer is recommended.

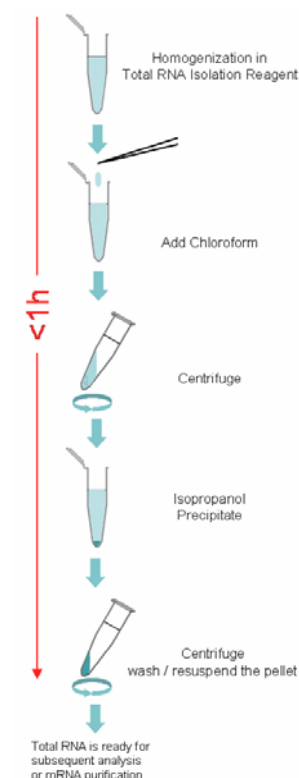
1. Homogenize tissue (50 mg) in 1 ml of Total RNA Isolation Reagent.

**From fresh tissue:** Immediately after dissection, weigh tissue and add the Total RNA Isolation Reagent.

**From frozen tissue:** Weigh frozen tissue and homogenise directly in Total RNA reagent.

2. Tranfert the lysate in a microtube and centrifuge at 10000 x g for 5 min
3. Transfer the supernatant in a tube and add 0.1ml of chloroform, mix well (shake vigorously 15-30 s) and incubate on ice for 5 min.
4. Follow the procedure at the step A 2.

### PROTOCOL OVERVIEW



### STORAGE / STABILITY

When stored in unopened vials at 2-8°C, Total RNA Isolation Reagent is stable until expiration date printed on the label.

### PRECAUTIONS

Precautions should be taken to prevent RNase contamination. Work in an RNase free environment. Wear gloves in order to avoid contamination from hands.

The Total RNA Isolation reagent is toxic and contains phenol (T,C poison) and guanidine thiocyanate (irritant, Xn). Avoid any exposure and read the MSDS before starting.

### WARNINGS AND LIMITATIONS

**For research use only.** Not for use in human diagnostic or therapeutic procedures.

### WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life. Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

Ademtech SA - Parc scientifique Unitec 1 - 4, allée du Doyen G. Brus - 33600 PESSAC - FRANCE  
www.ademtech.com