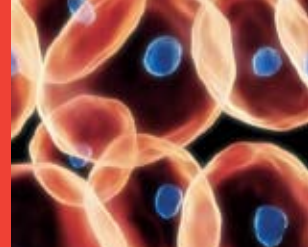


# Thermo Scientific CytosALL™ Reagent for Rapid RNA Preparation



## INSTRUCTIONS FOR USE

This protocol is designed for use with eukaryotic cells grown in monolayer or suspension culture. The number of cells can be adjusted up or down as needed, however it is important to maintain the ratio of reagent to cells at 600  $\mu\text{l}/10^6$  cells.

### Prepare the cell pellet:

1. Harvest and pellet  $1 \times 10^6$  cultured cells. Place on ice.
2. Wash once with cold phosphate buffered saline (PBS).
3. Remove and discard supernatant PBS.

### Prepare lysis reagent:

1. For each  $1 \times 10^6$  cell pellet, add 6  $\mu\text{l}$  RNase inhibitor (20-40 units/ $\mu\text{l}$ ) to 600  $\mu\text{l}$  CytosALL Reagent.
2. Note: The number of cells can be adjusted up or down as needed, however it is important to maintain the ratio of reagent to cells at 600  $\mu\text{l}/10^6$  cells.

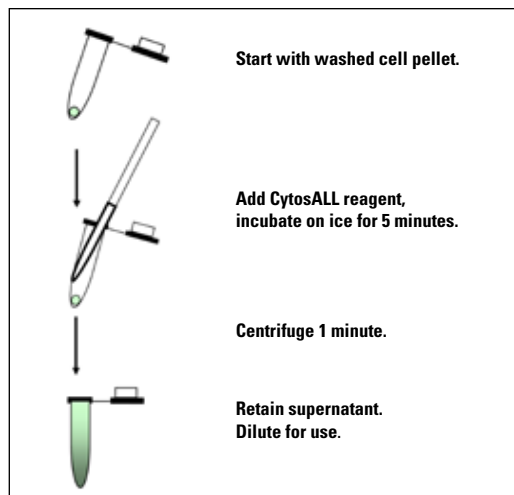
### Lyse the cells:

1. Add 600  $\mu\text{l}$  cold lysis reagent to the pellet.
2. Resuspend the pellet by pipetting eight to ten times or until the pellet is completely disrupted.
3. Incubate on ice for five minutes.

### Recover the cytoplasmic lysate:

1. Centrifuge the cell lysate at 4°C for one minute at 15,000 x g. While the cell lysate is centrifuging, place a new microcentrifuge tube on ice.
2. Immediately transfer the supernatant to the new chilled microcentrifuge tube.

Aliquot and store the undiluted lysate at -20°C or -80°C (for up to two weeks) or use directly in desired application.



	Suggested Dilution Range	Volume to add to 20 $\mu\text{l}$ RT reaction	Volume to add to 25 $\mu\text{l}$ PCR reaction	Cell equivalents
<b>1-step RT-PCR</b>	1:2 – 1:1,000	–	1 $\mu\text{l}$ diluted sample	1 – 1,000
<b>1-step qRT-PCR</b>	1:75 – 1:15,000	–	5 $\mu\text{l}$ diluted sample	<0.5 – 100*
<b>2-step RT-PCR</b>	Neat – 1:20,000	1 $\mu\text{l}$ diluted sample	1 – 2.5 $\mu\text{l}$ RT reaction	1 – 400
<b>2-step qRT-PCR</b>	1:5 – 1:50,000	1 $\mu\text{l}$ diluted sample	1 – 5 $\mu\text{l}$ RT reaction	<0.5 – 50*

Some applications may require dilution of the lysate. The table above provides general recommendations for dilution; the optimal dilution will need to be determined experimentally.