REzol™ C & T

Cat # : PT-KP200CT Size : 200 mL

Product Description:

REzol™ C & T is a ready-to-use reagent for the isolation of total RNA from cells and tissue. The REzol™ C & T RNA isolation method for total RNA is a new improved single-step method and modified from acid guanidine-thiocyanate-phenol-chloroform extract procedure of Chomczynski and Sacchi. The principles for RNA isolation by REzol™ C & T are cell disruption, RNase inactivation and deproteinization of the RNA, and physical separation of the RNA from other macro-molecules. At the acid *p*H in REzol™ C & T RNA isolation, the solubility of DNA and protein are decreased and partition to the organic phase and interphase, whereas the RNA remains exclusively in the aqueous phase.The total RNA is precipitated from the aqueous phase by addition of isopropanol, wash with ethanol and solubilized in DEPC-treated water.

The entire procedure for RNA isolation using the REzol™ C & T method can be completed in less than 1 hour. The total RNA isolated by REzol™ C & T method is undegraded and free of protein and DNA contamination as shown in Figure 1. The simplicity of REzol™ C & T method and excellent recovery of RNA makes this product especially suitable for RT-PCR, northern blot analysis, dot blot hybridization, poly (A) selection, *in vitro* translation, molecular cloning, and RNase protection assay.

Components: *Store at 4 °C*

1 bottle of 200mL REzol™ C & T RNA isolation solution

components not supplied:

chloroform, isopropanol, 75% ethanol (in DEPC-treated water), DEPC-treated water.

General Protocol:

1. Homogenate of cells or tissues are prepared in various ways:

- Suspension cells:

  Pellet 5 x 10^6 to 1 x 10^7 cells in microfuge tube, remove and discard supernatant, Lyse cells thoroughly in 1 mL REzol™ C & T in the shortest time possible by vortexing or repetitive pipetting.

- Monolayer cells:

  Remove and discard the culture fluid. Lyse cells by adding directly to the culture dish or flask 1 mL REzol™ C & T per 10 cm^2 dish.

	3.5 cm dish	6 cm dish	10 cm dish	T25 flask
Area	9.6 cm^2	28 cm^2	78.5 cm^2	25 cm^2
REzol™ Amount	960 uL	2.8 mL	7.85 mL	2.5 mL

- Tissues:

  Homogenize 10-100 mg of tissue in 1 mL of REzol™ C & T with a few strokes in a hand-held glass-Teflon or power homogenizer.

2. Transfer the cell lysate to a microfuge tube, and incubate the homogenate for 5 minutes at room temperature.
3. Add 0.2 mL of chloroform per 1 mL of REzol^{TM} C & T. Cover the samples tights and shake vigorously for 15 seconds and incubate them at room temperature for 2 minutes.
4. Centrifuge the samples at 12,000x*g* for 15 minutes at 4℃. After centrifugation, the homogenate form two phase: the lower red phenol-chloroform phase and the colorless upper aqueous phase. The RNA remains exclusively in the aqueous phase (with a volume of approximately 0.6 mL), whereas the DNA and proteins remain in the interphase and organic phase.
5. Carefully transfer the aqueous phase (0.5 mL) to a new microfuge tube without disturbing or touching the interphase. Add an equal volume (0.5 mL) of isopropanol and mix gently.
6. Incubate the samples for 10 minutes at room temperature and centrifuge them at 12,000 x*g* for 10 minutes at 4℃. The RNA precipitate (often invisible before centrifugation) forms a white pellet at the bottom of the tube.
7. Carefully remove the supernatant, wash the RNA pellet in 1 mL of 75% ethanol by vortex-mixing and subsequence centrifugation at 12,000 x*g* for 5 minutes at 4℃.
8. Carefully remove the ethanol and dry the pellet by air or under vacuum. **Do not dry the RNA by vacuum.** It is important not to over-dry the pellet because this makes it harder to dissolve the RNA.
9. Dissolve the RNA pellet in 20-100 uL of DEPC-treated water or in DEPC-treated 0.5% SDS byt repetitive pipetting. Store the samples for up to 1 year at -70℃.

Notes:

a. Materials, hands and dust may be the major source of RNase contamination. Always wear disposable gloves and keep tubes closed. The use of sterile, disposable polypropylene tubes is recommended through the procedure.
b. After homogenization, cells can be stored in REzol^{TM} C & T at -70℃ for at least 1 month.
c. Cells should not be washed with PBS.
d. Complete homogenization is crucial to obtain a large amount of high quality RNA that is free from proteins and DNA contaminations.
e. Leave 20% of the aqueous phase over the interphase.
f. The RNA precipitate can be stored in 75% ethanol at 4℃ for at least 1 week, or at least 1 year at -20℃.
g. Incubation of 10 minutes at 55-60℃ may be required to dissolve the RNA pellet.

Yield:

The total RNA isolated by REzol^{TM} C & T method is undegraded and free of protein and DNA contamination. The isolated RNA has an $A_{260/280}$ ratio of 1.7-2.0. The yield from 10^7 mammalian cells is 100-200 ug, and from 100mg of tissue, 150-500 ug, depending on the type of tissue.

Special handling precautions:

REzol^{TM} C & T contains an irritant (guanidine thiocyanate) and poison (phenol). When working with

REzol™ C & T, use gloves and eye protection. Use in a chemical fume hood. Avoid contact with skin. Avoid breathing vapor. Wash with large amount of water immediately in case of contact.

Trouble shooting:

1. Lower $A_{260/280}$ ratio
   Too small a volume of REzol™ C & T was used in sample homogenization.
   Over dried the RNA pellet or incompletely dissolved the RNA pellet.
   Contamination of aqueous phase with interphase/ organic phase.
2. RNA degradation
   Samples were not immediately processed or frozen.
   Samples or the isolated RNA preparations were stored at -5 to -20, instead of -60 to -70.
   Exogenous RNase contamination from hands, tubes, and dust.
   RNA-dissolving aqueous solutions were not RNase-free.

3. DNA contamination
   Contamination of aqueous phase with interphase/ organic phase.
   Too small a volume of REzol™ C & T was used in sample homonization.

Reference:

1. Chomczynski, P. and Sacchi, N. Single-Step Method of RNA Isolation by Acid Guanidine Thiocyanate-Phenol-Chloroform Extraction. Anal. Biochem. 162, 156-159. (1987)
2. Sambrook, J., Fritsch, R. F., and Maniatis, R. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989).

- For experimental sample, please test for your own conditions.
- REzol™ C & T is stable at 4°C for at least 1 year.

Research Use Only

Please do not hesitate to contact us while you have any questions.

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