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I. Description

AccuZol™ is a ready-to-use reagent for the isolation of total RNA from tissues and cells.
A monophasic solution of phenol and guanidine-salt inhibits RNase activity, and so the reagent maintains integrity of the RNA during sample homogenization or lysis. The homogenate is separated into aqueous phase and organic phase by adding chloroform and centrifuging, and then RNA remains in the aqueous phase. After transfer of the aqueous phase, the RNA is precipitated with isopropyl alcohol, and it is washed with ethanol and solubilized. *AccuZol*™ performs well with small quantities of samples lager than ~10mg tissue or $1x10^6$ cells, of human, animal, plant, bacterial or viral origin. The procedure for RNA isolation can be completed in 1 hr. The isolated total RNA can be used for cDNA synthesis, RT-PCR, real-time RT-PCR, Northern/dot blot analysis, poly-A (+) selection, RNase protection assays and molecular cloning, etc.

II. Precautions for preventing RNase contamination

Ribonucleases (RNase) are very stable and extremely active enzymes that do not required co-factors. RNases are quite difficult to inactivate and small amounts are enough to destroy RNA. To minimize RNase contamination the following guidelines should be taken when working with RNA.

- Always wear latex or vinyl gloves and change gloves often. RNases arise readily from bacteria and molds present in dust and on skin and clothing.
- Use sterile, disposable plasticware to prevent cross-contamination with RNases from shared equipment.
- Non-disposable plasticware or glassware can be washed with detergent, rinsed several times with sterile distilled water, followed by thorough rinses with 0.1 N NaOH, 1mM EDTA and, finally, RNase-free sterile distilled water.
- Alternatively, glassware can be washed with detergent, well rinsed and baked in a dry oven at 240℃ for 4 or more hours. Please note that autoclaving will not completely inactivate all RNases. Glassware can be treated with DEPC (diethyl pyrocarbonate) by filling glassware with 0.1% DEPC, left to stand 12 hrs at 37℃ or more hours and then autoclaved to eliminate the DEPC.
- Electrophoresis tanks are cleaned with detergent, rinse several times with RNase-free water, rinsed with ethanol and allowed to dry.
- Aqueous solutions, including water, can be can be treated with 0.1% DEPC, left to stand 12 or more hours and then autoclaved.

III. Storage and handling

Store at 2-8℃. *AccuZol*™ has determined stability of 12 months from first-opening.
AccuZol™ is poisonous contact with skin will cause burns. After contact with skin, wash immediately with plenty of water and detergent.
Use gloves and eye protection when working with *AccuZol*™

IV. Reagent required, but not supplied

Chloroform
Isopropyl alcohol
80% Ethanol (in DEPC-treated water)
RNase-free water or 0.5% SDS solution

V. Average Yield of Total RNA Isolated from Various Samples

	Samples	Starting volume	Average yield (ug)
Animal Tissue	Liver Kidney Spleen Lung Brain	10 mg	50 - 70 25 - 40 40 - 60 15 - 25 8 - 12
Cultured Cell	HeLa A549 PC3	1 x 10^6 cells	15 - 30 15 - 25 15 - 25
Bacterial Cell	*E.coli*	1 x 10^7 cells	10 - 15
Plant Tissue	Bean leaf	100 mg	30 - 50
Blood	Human whole blood	250 ul	2 - 3

VI. Extperimental Protocol to RNA Isolation

1. Sample Preparation

- ***Tissue samples (fresh)***
  After dissection, homogenize immediately in *AccuZol*™ or freeze rapidly in liquid nitrogen.
- ***Tissue samples (frozen)***
  Weigh frozen tissue, break into a suitably sized piece, and homogenize directly in *AccuZol*™.
- ***Plant***
  Weigh tissue, immediately place it in liquid nitrogen, and grind with a mortar and pestle.
  Fresh tissues can be homogenized directly in *AccuZol*™ with a mortar and pestle.
- ***Blood***
  Whole blood should be collected in the presence of an anti-coagulant. For optimal results, blood samples should be processed within a few hours of collection.

2. Homogenize prepared samples

- ***Tissue samples***
  Homogenize tissue samples in 10-20 volumes *AccuZol*™ (e.g., 1ml of *AccuZol*™ per 50-100mg of tissue) using a homogenizer or equivalent. The sample volume should not exceed 10% of the volume of *AccuZol*™ used for homogenization.
- ***Cell grown in monolayer***
  Lyse cells directly in the culture dish by adding 1ml of *AccuZol*™ per 10㎠ of culture dish area, and passing the cell lysate through a pipette several times. The amount of reagent added is based on the area of culture dish and not on the number of cells present.
- ***Cell grown in suspension***
  Pellet cells, then lyse in 1ml of *AccuZol*™ per 5-10x10^6 animal, plant, or yeast cells, or per 1x10^7 bacterial cells, by repetitive pipetting or vortexing. Disruption of some yeast and bacterial cells may require the use of a homogenizer.
- ***Plant***
  Homogenize plant samples in 1ml of *AccuZol*™ per 10-100mg of plant using a homogenizer or equivalent. The sample volume should not exceed 10% of the volume of *AccuZol*™ used for homogenization.
- ***Blood***
  For each 250ul of sample add 750ul of *AccuZol*™. Lyse cells in the sample suspension by passing the suspension several times through a pipette or vortexing.

3. Add 200ul of chloroform per 1ml of *AccuZol*™ and shake vigorously for 15 seconds.

4. Incubate the mixture on ice for 5 minutes.

5. Centrifuge at 12,000 rpm for 15 minutes at 4℃.

Following centrifugation, the mixture separates into a lower organic phase (green color), an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase.

6. Transfer the aqueous phase to a new 1.5ml tube and add equal volume of isopropyl alcohol.

When pipetting the upper layer, pay attention to form any white sediments.

7. Mix by inverting the tube 4-5 times and incubate at -20℃ for 10 minutes.

8. Centrifuge at 12,000 rpm for 10 minutes at 4℃, then carefully remove the supernatant.

9. Add 1ml 0f 80% ethanol and mix well by inverting or vortexing.

10. Centrifuge at 12,000 rpm for 5 minutes at 4℃, then carefully remove the supernatant.

11. Dry the pellet.

Do not dry the pellet by centrifugation under vacuum. Do not completely dry the RNA pellet as this will greatly decrease its solubility.

12. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60℃ (0.5% SDS should not be used).

RNA can also be in 100% formamide (deionized) and store at -70℃.

VII. Troubleshooting guide

- Low *yield*
  - Incomplete homogenization or lysis of samples.
  - Final RNA pellet incompletely redissolved.

- *Low A_{260}/A_{280} ratio (<1.6)*
  - The volume of *AccuZol*™ used for sample homogenization was too low.
  - The aqueous phase was contaminated with the phenol phase.
  - RNA pellet was not completely dissolved.
  - RNA sample was diluted in water instead of TE prior to spectrophotometric analysis.

- *RNA degradation*
  - Tissues were not immediately processed or frozen after removing from animal.
  - Samples used for isolation, or the isolated RNA were not stored long-term at -70℃.
  - Reagents or tubes were not RNase-free.

- *DNA contamination*
  - Sample was homogenized in too small a reagent volume.
  - Sample contained organic solvents (e.g., ethanol, DMSO), strong buffers or alkaline solution.

- *Proteoglycan & polysaccharide contamination*
  - The following modification of the RNA precipitation (step 6-8) removes these contaminating compounds from the isolated RNA.
  - Add 250ul of isopropanol to aqueous phase and 250ul of a high salt precipitation solution (e.g., 0.8M sodium citrate and 1.2M NaCl) per 1ml of *AccuZol*™ used for the homogenization.
  - Mix well and centrifuge at 12,000 rpm for 5-10 minutes at 4℃ and proceed with the isolation as described in the protocol.
  - When isolating RNA from plant sample containing a very high level of polysaccharide, additional centrifugation in step 5 is required to isolate pure RNA.