Triadzol™ RNA Kit

(Cat# K1353-4, -50, -100, -200; Store at RT)

I. Introduction:

Triadzol™ RNA Kit is a phenol and guanidine isothiocyanate plus spin column system for convenient purification of high-quality total RNA from a variety of samples. Initially, samples are homogenized in Triadzol™ Reagent without chloroform phase separation or isopropanol RNA precipitation. Following sample homogenization, simply bind, wash and elute the high-quality, total RNA in RNAse-free Water and use in a variety of sensitive downstream applications.

II. Application:

- cDNA Library Construction, Cloning, RT PCR (Endpoint), Real Time PCR, Nuclease Protection Assays, Northern Blotting

III. Key Features:

- Purify total RNA in 15 min
- A cost effective phenol, guanidine isothiocyanate solution plus spin column system
- No chloroform phase separation; No isopropanol RNA precipitation; No phenol carryover
- High quality RNA: A260/A280 >1.8; A260/A230 >1.8
- Binding Capacity: 50 µg RNA from ≥ 25 µl DNAse/RNAse-free Water
- Spin Columns: glass fiber membrane optimized for total RNA extraction (certified RNAse and DNAse-free)

IV. Sample Type:

Up to: 200 µl (blood, buffy coat, serum, plasma); $5x10^6$ cultured cells; 10-50 mg of tissue; $1x10^9$ bacterial cells; 20-50 mg of plant tissues

V. Kit Contents (Triadzol™ RNA Kit):

Components	K1353-4	K1353-50	K1353-100	K1353-200	Part Number
Triadzol™ Reagent	4 ml	40 ml	80 ml	160 ml	K1353-XX-1
Pre-Wash Buffer* (Add Ethanol)	1.4 ml (0.6 ml)	21 ml (9 ml)	35 ml (15 ml)	70 ml (30 ml)	K1353-XX-2
Wash Buffer** (Add Ethanol)	2 ml (8 ml)	25 ml (100 ml)	50 ml (200 ml)	25 ml + 50 ml (100 ml + 200 ml)	K1353-XX-3
RNAse-free Water	1 ml	6 ml	6 ml	15 ml	K1353-XX-4
RB Columns	4	50	100	200	K1353-XX-5
2 ml Collection Tubes	8	100	200	400	K1353-XX-6

,Add absolute ethanol (see the bottle label for volume) to Pre-Wash Buffer and Wash Buffer then mix by shaking for a few sec. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation*

VI. User Supplied Reagents and Equipment:

- Absolute Ethanol
- RNAse-free Water
- 1.5 ml microcentrifuge tubes (RNAse-free)
- Lysozyme and bacteria lysis buffer (bacteria only)
- 1 µL of 20 mM EGTA (pH=8.0) for Optional Step 2: DNA Digestion in Solution

VII. Shipment and Storage:

All the reagents are shipped at room temperature (2-25ºC) for up to atleast 12 months

VIII. Reagent Preparation and Storage Conditions:

- Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few sec

IX. RNA Extraction Protocol:

1. Sample Homogenization and Lysis:

Sample preparation should be performed at room temperature. Please follow the table below for specific sample preparation. To avoid DNA contamination of extracted RNA, be sure and use the indicated volume of Triadzol™ Reagent

Adherent Cultured Cells

- Remove the culture medium from the culture dish
- Directly add 100 µl of Triadzol™ Reagent per cm^2 of culture dish surface area
- Lyse the cells directly in the culture dish by pipetting several times
- Incubate the sample mixture for 5 min at room temperature
- Transfer the sample to a 1.5 ml microcentrifuge tube (RNAse-free)

Suspension Cultured Cells

- Transfer cells (up to $5x10^6$) to a 1.5 ml microcentrifuge tube (RNAse-free)
- Harvest by centrifugation at 300g for 5 min then remove the culture medium completely
- 700 µl of Triadzol™ Reagent should be added to the cell pellet then mixed several times by pipette
- Incubate the sample mixture for 5 min at room temperature

Tissue

- Excise 10-50 mg of tissue directly from the animal or remove the tissue sample from storage. Do not use more than 50 mg of tissue per reaction
- Homogenize tissue samples using one of the following methods: A. Transfer the tissue and 700 µl of Triadzol™ Reagent to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. B. Transfer the tissue and 700 µl of Triadzol™ Reagent to a 1.5 ml centrifuge tube and grind the tissue with a micropestle a few times then shear the tissue by passing the lysate through a 20-G needle syringe 10 times. C. Transfer the tissue and 700 µl of Triadzol™ Reagent to a glass-Teflon or Polytron homogenizer. Transfer the homogenized sample to a 1.5 ml microcentrifuge tube (RNAse-free)
- Incubate the homogenized sample for 5 min at room temperature

Body Fluids (blood, buffy coat, plasma, serum)

- Transfer up to 200 µl of liquid sample to a 1.5 ml of microcentrifuge tube (RNAse-free)
- Add 3 vol of Triadzol™ Reagent per 1 volume of sample (3:1) then mix well by vortex
- Incubate the sample mixture for 5 min at room temperature

Bacteria

- Transfer bacteria cells (up to $1x10^9$) to a 1.5 ml microcentrifuge tube (RNAse-free)
- Centrifuge at 12-16,000g for 2 min then remove the supernatant completely

- Weigh and transfer 10 mg of lysozyme powder to a new 1.5 ml microcentrifuge tube (RNAse-free)
- Add 1 ml of bacteria lysis buffer to the microcentrifuge tube containing 10 mg of lysozyme
- Vortex the tube until the lysozyme powder is completely dissolved
- Add 100 µl of bacteria lysis buffer containing lysozyme to the bacteria cell pellet
- Resuspend the cell pellet by vortex or pipetting. *NOTE: Residual bacteria lysis buffer containing lysozyme should be stored at 4ºC for 2 weeks*
- Incubate the sample for 5 min at room temperature
- Add 700 µl of Triadzol™ Reagent, mix well by pipette then incubate at room temperature for 5 min

Plant

- Cut off 20-50 mg of fresh or frozen plant tissue. Do not use more than 50 mg of plant tissue per rxn
- Homogenize plant tissue samples using one of the following methods: A. Transfer the plant tissue and 700 µl Triadzol™ Reagent to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. B. Add liquid nitrogen to a mortar (RNAse-free) and grind the plant tissue thoroughly using a pestle (RNAse-free). Transfer the plant tissue powder and 700 µl of Triadzol™ Reagent to a 1.5 ml centrifuge tube then vortex briefly
- Incubate the homogenized sample for 5 min at room temperature

2. RNA Binding:

- Centrifuge the sample at 12-16,000g for 1 min to remove cell debris then transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (RNAse-free). *NOTE: When extracting RNA from cultured cell samples, cell debris will not commonly collect on the bottom of the microcentrifuge tube. In this case, proceed without transferring the supernatant*
- Add 1 vol of absolute ethanol directly to 1 volume of sample mixture (1:1) in Triadzol™ Reagent
- Mix well by vortex then place a RB Column in a 2 ml Collection Tube
- Transfer 700 µl of the sample mixture to the RB Column. Centrifuge at 14-16,000g for 1 min then discard the flow-through
- Repeat the RNA Binding Step by transferring the remaining sample mixture to the RB Column
- Centrifuge at 14-16,000g for 1 min then discard the flow-through. Place the RB Column in a new 2 ml Collection Tube

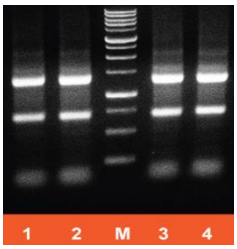
3. RNA Wash:

- Add 400 µl of Pre-Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000g for 30 sec
- Discard the flow-through then place the RB Column back in the 2 ml Collection Tube
- Add 600 µl of Wash Buffer (make sure ethanol was added) to the RB Column
- Centrifuge at 14-16,000g for 30 sec then discard the flow-through. Place the RB Column back in the 2 ml Collection Tube
- Add 600 µl of Wash Buffer (make sure ethanol was added) to the RB Column
- Centrifuge at 14-16,000g for 30 sec then discard the flow-through
- Place the RB Column back in the 2 ml Collection Tube. *NOTE: For blood samples only, wash the RB Column again with 600 µl of Wash Buffer*
- Centrifuge at 14-16,000g for 3 min to dry the column matrix

4. RNA Elution:

- Place the dry RB Column in a clean 1.5 ml microcentrifuge tube (RNAse-free)
- Add 25-50 µl of RNAse-free Water into the CENTER of the column matrix
- Let stand for at least 3 min to ensure the RNAse-free Water is completely absorbed by the matrix
- Centrifuge at 14-16,000g for 1 min to elute the purified RNA

X. Functional Data:



Test	RNA Conc.	260/280	260/230	Yield
1. Competitor Z	162.5 µg/ml	2.00	2.07	8.1 µg
2. Competitor Z	160.7 µg/ml	2.03	2.07	8.0 µg
3. BioVision	164.0 µg/ml	2.00	2.07	8.2 µg

Figure 1. RNA was purified using the Triadzol™ RNA Kit in parallel to the similar product from competitor Z. $5x10^5$ HeLa cells were homogenized using Triadzol™ Reagent and competitor Z reagent. RNA was then purified using the corresponding kits spin column procedure. 10 µl from a 50 µl eluate of purified RNA was analyzed by electrophoresis on a 0.8% agarose gel.

XI. Related Products:

Product Name	Cat. No.
RNA Mini Kit (Blood/Cells)	K1340
RNA Mini Kit w/DNAse (Blood/Cells)	K1341
Plant RNA Mini Kit	K1342
Plant RNA Mini Kit w/DNAse	K1343
Tissue RNA Mini Kit	K1344
Tissue RNA Mini Kit w/DNAse	K1345
Alk Lys™ Bacterial RNA Kit	K1346
Alk Lys™ Bacterial RNA Kit w/DNAse	K1347
Alk Lys™ Yeast RNA Kit	K1348
Alk Lys™ Yeast RNA Kit w/DNAse	K1349
miRNA Purification Kit	K1350
Triadzol™ Reagent	K1351
Triadzol™ Bacterial RNA Kit	K1352
Triadzol™ RNA Kit	K1353

Triadzol™ RNA Kit w/DNAse	K1354
Phase Free RNA Kit	K1355
Phase Free RNA Kit w/DNAse	K1356
RNA Washup Kit	K1357
96 Well Phase Free RNA Kit	K1358

XII. General Troubleshooting Guide:

Problem	Cause	Solution
Low Yield	A. Sample lysis or homogenization was incomplete B. Incorrect RNA elution C. Precipitates may form during the RNA binding step after adding 1 vol of absolute ethanol to the sample mixture in Triadzol™ Reagent if too much sample material is used	A. Starting material should be reduced and completely dissolved in Triadzol™ Reagent B. Make sure RNAse-free Water is added to the center of the RB Column and is absorbed completely C. Reduce the sample amount to half of the original
Degraded RNA	A. Incorrect sample preparation and/or final storage B. Incorrect sample storage temperature	A. Process or freeze samples immediately after collection. B. Extracted RNA should be stored at -70ºC
Low RNA A260/A280	A. Volume of Triadzol™ Reagent was insufficient for proper sample homogenization B. Incomplete wash step	A. Volume of Triadzol™ Reagent is sample dependent and should be added according to the sample homogenization specifications B. Wash the RB Column with ethanol added Wash Buffer 3 times
Eluted RNA does not perform well in downstream applications	A. Residual phenol contamination	A. Following the wash step, dry the RB Column with additional centrifugation at 14-16,000g for 5 min or incubate at 60°C for 5 min
Samples were stored in 1 ml of tri-reagent in a 1.5 ml microcentrifuge tube	A. 1 ml (1 vol) of absolute ethanol cannot be added to the same 1.5 ml microcentrifuge tube	A. Following centrifugation to remove insoluble cell debris, transfer the supernatant to a 2 ml or 15 ml centrifuge tube (RNAse-free) and add 1 volume of absolute ethanol then mix well by vortex. Transfer 700 µl of the sample mixture to the RB Column then centrifuge and discard the flow through. Repeat the RNA Binding step until all of the sample mixture has been passed through the RB Column

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