Tissue RNA Mini Kit w/DNAse

(Cat# K1345-4, -50, -100, -300; Spin Column Based; Store at multiple temperatures)

I. Introduction:

The Tissue RNA Mini Kit w/DNAse was designed specifically for purifying total RNA from a variety of animal and paraffin-embedded tissue. Tissue samples can be efficiently homogenized in a microcentrifuge tube using the provided micropestle. Detergents and chaotropic salt are used to lyse cells and inactivate RNAse with an optional in-column DNAse treatment. RNA in the chaotropic salt is bound by the glass fiber matrix of the spin column. Once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNAse-free Water.

II. Application:

- RT-PCR, Northern Blotting, Primer Extension, mRNA Selection, cDNA Synthesis, RNAse Protection Assay

III. Key Features:

- Purify total RNA within 15 min
- High Yield: 5-30 µg of pure RNA
- RNAse and DNAse-free Micropestles included for simple tissue homogenization within the tube to reduce the risk of sample contamination
- Spin Columns: glass fiber membrane optimized for total RNA extraction
- No phenol extraction or alcohol precipitation
- Individually packaged RNA spin columns and collection tubes, certified RNAse and DNAse-free

IV. Sample Type:

- Variety of animal tissues and paraffin-embedded tissue (up to 25 mg of tissue including tail snips, liver, kidney, brain, spleen, ear punches etc.; up to 25 mg of paraffin-embedded tissue)

V. Kit Contents (Tissue RNA Mini Kit):

Components	K1345-4	K1345-50	K1345-100	K1345-300	Part Number	Storage Temp
RB Buffer	2 ml	30 ml	60 ml	130 ml	K1345-XX-1	RT
DNAse I (2U/µl)*	20 µl	275 µl	550 µl	550 µl x 3	K1345-XX-2	RT
DNAse I Reaction Buffer	200 µl	2.5 ml	5 ml	15 ml	K1345-XX-3	-20ºC
W1 Buffer	2 ml	30 ml	50 ml	130 ml	K1345-XX-4	RT
Wash Buffer (Add Ethanol)**	1.5 ml (6 ml)	25 ml (100 ml)	25 ml (100 ml) +12.5 ml (50 ml)	50 ml x 2 (200 ml x 2)	K1345-XX-5	RT
RNAse-free Water	1 ml	6 ml	15 ml	30 ml	K1345-XX-6	RT
Filer Columns	4	50	100	300	K1345-XX-7	RT
RB Columns	4	50	100	300	K1345-XX-8	RT
2 ml Collection Tubes	8	100	200	600	K1345-XX-9	RT
Micropestles	4	50	100	300	K1345-XX-10	RT

**DNAse I is shipped at room temperature and should be stored at -20ºC after receiving the kit. **Add absolute ethanol (see the bottle label for volume) to 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:

- Pipettes
- PBS
- 20-G needle syringe
- Xylene (FFPE)
- Absolute ethanol
- ddH_2O (RNAse/DNAse-free)
- Microcentrifuge tubes
- Pipette tips
- ß-mercaptoethanol

VII. Shipment and Storage:

All the reagents are shipped and stored at room temperature (15-25ºC) for up to atleast 9 months without showing any reduction in performance. DNAse I is shipped at room temperature and should be stored at -20ºC after receiving the kit

VIII. Reagent Preparation and Storage Conditions:

- Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
- To prevent RNAse contamination, disposable and non-disposable plasticware and automatic pipettes should be sterile and used only for RNA procedures

IX. RNA Mini Kit (Tissues) Protocol:

Sample Preparation:

Paraffin-Embedded Tissue:

- Slice up to 25 mg from a block of FFPE tissue and transfer to a 1.5 ml microcentrifuge tube and add 1 ml of xylene\.
- Vortex vigorously then incubate at room temperature for approximately 10 min. *NOTE: Vortex occasionally during incubation*
- Centrifuge at 14-16,000g for 3 min to form a pellet then remove the supernatant
- Add 1 ml of absolute ethanol to wash the sample pellet then mix by inverting
- Centrifuge at 14-16,000g for 3 min then remove the supernatant
- Add 1 ml of absolute ethanol to wash the sample pellet again then mix by inverting
- Centrifuge at 14-16,000g for 3 min then remove the supernatant
- Open the tube and incubate at 37ºC for 15 min to evaporate ethanol residue then proceed with Cell Lysis

Fresh or Frozen Animal Tissue:

• Cut off up to 25 mg of fresh/frozen tissue and transfer to a 1.5 ml microcentrifuge tube then proceed with Cell Lysis. *NOTE: If using frozen animal tissue, the sample must have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use to avoid RNA degradation*

Step 1/Cell Lysis:

- Add 400 µl of RB Buffer and 4 µl of ß-mercaptoethanol to the 1.5 ml microcentrifuge tube
- Use the provided Micropestle to grind the tissue or FFPE tissue pellet a few times

- Shear tissue by passing lysate through a 20-G needle syringe 10 times then incubate at room temp for 3 min
- Place a Filter Column in a 2 ml Collection Tube and transfer the sample mixture to the Filter Column
- Centrifuge for 30 sec at 1,000g then discard the Filter Column
- Carefully transfer the filtrate to a new 1.5 ml microcentrifuge tube

Step 2/RNA Binding:

- Add 400 µl of 70% ethanol prepared in ddH_2O (RNAse and DNAse-free) and shake the mixture vigorously. NOTE: If precipitate appears, break it up as much as possible with a pipette
- Place a RB Column in a 2 ml Collection tube then transfer the mixture to the RB Column
- Centrifuge at 14-16,000g for 1 min. *NOTE: If the lysate mixture could not flow past the RB Column membrane following centrifugation, increase the centrifuge time until the lysate mixture passes completely*
- Discard the flow through then place the RB Column back in the 2 ml Collection Tube

In Column DNAse I Digestion:

The amount of DNA contamination is significantly reduced following In Column DNAse I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2, DNA Digestion In Solution to efficiently remove trace amounts of DNA. Standard DNAse buffers are incompatible with In Column DNAse I Digestion and may effect RNA integrity and reduce yield

1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000g for 30 sec
2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube
3. Prepare DNAse I solution in a 1.5 ml microcentrifuge tube (RNAse-free) as follows:

DNAse I	5 µl (2 U/µl)
DNAse I Reaction Buffer	45 µl
Total Volume	50 µl

4. Gently pipette DNAse I solution to mix (DO NOT vortex) then add DNAse I solution (50 µl) into the CENTER of the RB column matrix
5. Incubate the column for 15 min at room temperature (20-30ºC) then proceed with Step 3 RNA Wash

DNA Digestion In Solution:

1. Prepare DNAse I reaction in a 1.5 ml microcentrifuge tube (RNAse-free) as follows:

RNA in RNAse-free Water	1-40 µl
DNAse I	0.5 µl/µg RNA
DNAse I Reaction Buffer	5 µl
RNAse-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2. Gently pipette the DNAse I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 min
3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65°C for 10 min
4. Repurify the RNA sample by adding 5 volumes of RB Buffer to the DNAse I reaction (e.g. 250 µl of RB Buffer to 50 µl of DNAse I reaction) then mix well by vortex. Add 1 volume of 70% ethanol to 1 volume of sample mixture then mix well by vortex. Transfer all of sample mixture to a new RB Column. Centrifuge at 14-16,000g for 1 min. Discard the flow through. Proceed with Step 3 RNA Wash

Step 3/Wash:

- Add 400 µl of W1 Buffer 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) into 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) into the RB Column
- Centrifuge at 14-16,000g for 30 sec
- Discard the flow-through then place the RB Column back in the 2 ml Collection Tube
- Centrifuge at 14-16,000g for 3 min to dry the column matrix

Step 4/RNA Elution:

- Place the dried RB Column in a clean 1.5 ml microcentrifuge tube
- Add 50 µl of RNAse-free Water to the CENTER of the column matrix
- Let stand for at least 2 min to ensure the RNAse-free Water is absorbed by the matrix
- Centrifuge at 14-16,000g for 1 min to elute the purified RNA

X. Functional Test Data:

Total RNA was extracted from 10 mg of various mouse tissue using the Tissue RNA Mini Kit (Tissue). 5 µl aliquots of each sample was analyzed by electrophoresis on a 1% agarose gel. 1. Brain, 2. Lung, 3. Liver, 4. Spleen, 5. Kidney.

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	Possible Reasons/Solution
Clogged Column	• Insufficient disruption and/or homogenization • Too much starting material • Centrifugation temperature was too low (should be 20ºC-25ºC)
Low RNA Yield	• RNA still bound to the RB Column membrane • Ethanol carryover • Insufficient disruption and homogenization/too much starting material
RNA Degradation	• Harvested sample not immediately stabilized • Inappropriate handling of starting material • RNAse contamination

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