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PRODUCT INFORMATIOIN

RNA isolation Kit for Total RNA (>200nt) from Animal/Human Cell and Animal Tissue (Cat#EZCR300)

Kit Contents:

Components	EZCR300	Storage
RL Buffer*	25ml	18 months at room temperature (except, RL Solution should be kept at 2-8^{0}C). For longer storage, keep all contents at 4^{0}C.
WASH 1 Solution** (concentrated)	18ml	
WASH 2 Solution** (concentrated)	6ml	
DEPC-Water (RNase-free)	5ml	
RNA Mini Spin Column (with collection tubes)[a]	50	
Shredder Spin Column (with collection tube)[b]	50	

(*) RL Solution should be kept at 2-8^{0}C. It may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37^{0}C.
(**) WASH 1 & 2 Solutions: Before use add **12ml** 100% ethanol to 18 ml WASH1 and **24ml** 100% ethanol to 6ml WASH2.
a,b: Mini Spin column (EZCR101) and shredder column (EZC109) are sold separately for leftover solutions.

Reagents and equipment supplied by the user

- RNase-free Ethanol (100%)
- Centrifuge for micro-centrifuge tubes
- RNase-free micro-centrifuge tubes
- Manual micro-pipettors and sterile, RNase-free tips
- Equipment for sample disruption

Principle:

This kit is designed for fast isolation of high quality intact total RNA (>200nt) from cultured cells, animal tissues; blood sample and for RNA cleans up as well. The reagent contains disruptive and protective properties of guanidine isothiocyanate and β–mercaptoethanol to inactivate the ribonucleases present in cell extracts. The kit contains a membrane embedded spin column for binding up to 20 µg of RNA and a shredder spin column for lysate homogenization. Nucleotides, proteins, salts, and other impurities do not bind to the EZ-minispin Column and Shredder Spin Colum. The total RNA is eluted from the membrane in RNase-free water. The recommended sample size is <5x10^6 cell and <20mg tissue. For small sample <5x10^5 cell and <5mg tissue, please use our RNA micro Kit (cat# 700).

Features:

√ Fast (20 min procedure) and High quality (OD_{260}/OD_{280} ratio>1.9) using RNA spin column format.
√ No phenol / chloroform extraction or ethanol precipitation needed √ Columns (Cat#EZCR101) are sold separately for leftover solutions

Note: Care must be taken when working with RNA. It is important to maintain an RNAse-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNAse free tubes, tips, gels. Wear gloves at all times. Change gloves frequently to avoid contaminating samples with RNases.
RNA is exposed to RNA-degrading enzymatic activity until the sample is frozen or disrupted using RNase-inhibiting agents. Plant and animal tissue samples should be flash frozen in liquid nitrigen immediately and stored at –70°C or processed as soon as possible.

Procedures for Isolation of Total RNA from Animal and Human Cells

Important Notes:

In order to obtain optimal RNA yield and purity, it is essential to use the correct amount of starting material and the amount of lysis solution (RL) for efficient cell lysis. Here is the recommendation for different sample types:

RL Solution	Number of Cells	Fresh Tissue	Tissue stored in RNAlater	Fatty tissue (brain and Adipose tissue)
175 µl	<1x10^5 (48 well plate)	<5mg		
350 µl	<5x10^6 (<60mm dish)	<20mg	<20mg	20mg
600 µl	5x10^6-1x10^7(60-100mm dish)	20-30mg	20-30mg	

*If 600 µl RL Buffer and ethanol are used, sample must be loaded onto the column in two successive centrifugation steps.

Cell numbers in Different Sizes of Multiwall Culture Plates and Dishes

Multiwell plates	Number of Cells	Culture Dishes	Number of Cells
96-well	4-5x10^4	35mm dish	1x10^6
48-well	1x10^5	60mm dish	2.5x10^6
24-well	2.5x10^5	100mm dish	7x10^6 to 1x10^7
12-well	5x10^5	145-150mm	2x10^7
6-well	>1x10^6	40-50ml Flask	3x10^6
		250-300 ml Flask	1x10^7
		650-750ml Flask	2x10^7

1. Samples Preparation (**<5x10^6** Cells)
   a) Count cells; pellet up to 5x10^6 cells by centrifugation, and thoroughly remove supernatant by aspiration.
   b) The cell pellet can be washed with 1X phosphate buffered saline (PBS) prior disruption, but this is not essential. To wash cells in PBS, resuspend in 1 mL PBS, and pellet the cells, and thoroughly remove the fluid.
   c) Proceed immediately to the next step, sample disruption.

B. Disrupt cells by addition of RL Solution*:
   a) Add 350 µl RL Solution to the cell pellet (cell <5x10^6).
   b) Resuspend the pellet by vortexing/pipetting. No cell clumps should be visible before proceeding to Step 2.

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

2. Homogenization of lysate:

A. Add the lysate mixture from step 1 to Shredder Spin Columns (also sold separately EZC109) and spin at 14,000xg for 1minute and transfer the flow-through to a new 1.65ml DNase/RNase free microcentrifuge tube (EZS165NS, $35 for1000/pk).

B. (Option) If higher number of cells (>1x10^6) or tissue (>30mg) must be processed, the lysate should be homogenized by passing through a 25 gauge needle 5 times, followed by filtration through shredder spin column.

Note: If processing <1x10^5 Cell, the lysate can be homogenized by vortexing without shredder spin column. For small amount RNA preparation, please use our RNA micro Kit (cat# 700).

3. Add an equal volume of 70% ethanol (350 or 450µl) to the homogenized lysate (flow-through from step 2A), and mix by inverting the tube.

Note: precipitate may form after adding ethanol, but this will not affect the procedure. Load all of the precipitate on the column as described in step 4.

4. Transfer above ethanol mixture to RNA Mini spin column, centrifuge at 13,000rpm (~11,000 x g) for 1 minute. Discard the flow-through or save the flow-through if you want to recover small RNA (includes miRNA, 5S rRNA, and tRNA), for details, see Option 2 below.

Note: Maximal loading capacity of the RNA mini spin column is 750µl. Repeat the step 4 if more than 750µl is processed.

5a. Discard the flow-through. Add 400 µl of WASH 1 Solution to the Spin Column and spin at 13,000 rpm (>11,000 x g) for 1 minute. Discard flow-through and place the column back to the same Collection Tube.

5b. On-column rDNase treatment if needed.

Option: Base on your application, On-column rDNase 1 (EZrDNase1 set sold separately) treatment will eliminate genomic DNA contamination (See procedure from **Option**).

6. Add 500 µl of WASH 2 Solution to the spin column, spin at 13,000 rpm (>11,000 x g) for 1 minute. Discard the flow-through

7. Cut off the lid of the spin column and centrifuge for 2 min at full speed to remove residue of WASH 2 Solution. This step is very important to remove the residual ethanol thoroughly.

8. Transfer the spin column to a clean RNase-free 1.65 ml microtube. Add 50 µl of RNase-free water to the center part of the column; incubate at room temperature for 2 minutes. Spin to elute the RNA at full speed for 1 minute. RNA is ready for use or kept at –80^{0}C for long term storage.

Note: Do not elute with less than 20 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated. For small amount RNA preparation, please use our RNA micro Kit (cat# 700). The elution volume for RNA Tini spin column (EZC107) can be as low as 5µl.

Procedure for Isolation of Total RNA from Animal Tissues

1. Samples Preparation: It is essential to use correct amount of tissue. Excess sample may increase the risk of RNA degradation.

a. Immediately place the weighted tissue (20-30mg) to liquid nitrogen and grind thoroughly with mortar and pestle. Transfer the tissue power with liquid nitrogen to a dry ice pre-chilled RNase-free microcentrifuge tube. Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

b. Add 350µl RL solution to the tissue sample, vortex and pipet the lysate into EZ shredder and spin for 2min at full speed.

2. Collect the flow-through and add ethanol: follow the step 3-8 from the protocol above.

Note:

Fatty tissue (brain+adipose tissue): Lysis Additive (cat# LA101) is required during the lysis step.

Fibrous tissues (skin, skeletal muscle, heart): Proteinase K treatment is recommended during the lysis step.

Procedure for Isolation of RNA from Blood

This kit is designed to isolate RNA from non-coagulating fresh blood (using EDTA as the anti-cogulant). It is recommended that no more than 100 µl of blood be used in order to prevent clogging of the column.

1. Sample preparation and homogenization:

Transfer up to 100µl non-cogulating blood to an RNase-free microcentrifuge tube. Add 350µl of RL buffer to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step. Shredder spin column can be used to filter the lysate if necessary (see step 2 in the first protocol).

2. Add ethanol: follow the rest of the step 3-8 from the first protocol above.

Procedure for Isolation of RNA from Nasal or Throat Swabs

1. Sample preparation and homogenization:

a. Add 450 µl RL buffer to an RNase-free microcentrifuge tube. Cut the cotton tip where the nasal or throat cells were collected under sterile condition, and place the tip into the tube with RL buffer. Close the tube and vortex gently and incubate for 5 minutes at room temperature.

b. Transfer the lysate into anther RNase-free microcentrifuge tube. Record the volume of the lysate.

2. Add ethanol: follow the rest of the step 3-8 from the first protocol above.

Procedure for Isolation of RNA from Paraffin Embedded Tissue

1. Sample preparation

a. Place 10mg of finely minced tissue into a 1.5ml microcentrifuge tube.

b. Add 300 µl Xylene and incubate at room temperature for 5 min with constant mixing.

c. Centrifuge at 13000 rpm in microcentrifuge for 3 min to pellet the tissue and discard the Xylene.

d. Repeat step b and c twice for total of 3 Xylene washes.

e. Add 300 µl of 96% ethanol and incubate at room temperature for 5 min with constant mixing

f. Centrifuge at 13000 rpm in microcentrifuge for 3 min to pellet the tissue and discard the ethanol.
g. Repeat step e and f twice for total of 3 ethanol washes.
h. Grind sample under liquid nitrogen to a fine powder using a mortar or pestle. Transfer the mixture of tissue powder and liquid nitrogen to 1.5 ml microtube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Proceed immediately to Step 2.

2. Add 450 µl of RL Solution to the tissue powder and vortex vigorously. Incubation at 50^0C may help to disrupt the tissue sample.

3. Homogenization of lysate: follow the rest of the step 2-8 from the protocol above.

Procedure for RNA Cleanup

This procedure is to clean up or concentrate RNA that isolated by different methods or after enzymatic reactions.

1. Adjust sample to a volume of 50 µl with RNase-free H_2O, add 175 µl of RL Solution, and mix well.
2. Add ethanol:
   - For recovering only the large RNA: Add 125 µl of 100% ethanol to the sample and mix gently. A precipitate may form by adding ethanol, do not centrifuge, and proceed immediately to the next step.
   - For recovering both large and small RNA: Add 350 µl of 100% ethanol to the sample and mix gently. A precipitate may form by adding ethanol, do not centrifuge, and proceed immediately to the next step.
3. Place the Mini Spin Column in 2.0ml Collection Tube and transfer the mixture to the column and spin at 14,000 x g for 1 minute, discard flow-through.
4. Add 180 µl of WASH 2 Solution to the column and spin at 14,000 x g for 1 minute, discard the flow-through and spin once more to completely remove the residue of WASH 2 Solution.
5. Add 300 µl of 80% ethanol to the column and spin at 14,000 x g for 1 minute, discard the flow-through.
6. Cut off the lid of the spin column and centrifuge for 2 min at full speed to remove residue of ethanol. This step is very important to remove the residual ethanol thoroughly.
7. Add 50 µl of RNase-free water onto the center part of the membrane on the column and centrifuge at 14,000 x g for 1 minute. Keep RNA sample at -80^0C.

Option 1:

On column DNase (RNase free) treatment: Cat# EZrDNase1 set. In most case, this step is not necessary. However, for certain application that is sensitive to very small amount of DNA (e.g., TagMan RT-PCR analysis with a low-abundant target), and then DNase (RNase free) treatment can efficiently remove the DNA contamination.

Protocol:

Prepare rDNase1 stock solution: Inject or add 570ul of RNase-Free Water into rDNase vial, and mix by swirling (Do not vortex which will dramatically decrease the DNase 1 activity)—Aliquot the stock and keep at -80*C for up to 1-2 years. Avoid freeze thaw!
Prepare on column rDNase I cocktails:

- Tini Spin Column: Mix 3ul of rDNase1 stock with 21ul rDNase 1 reaction buffer gently by inverting the tube.
- Mini Spin Column: Mix 6ul of rDNase1 stock with 42ul rDNase 1 reaction buffer gently by inverting the tube.

1. (After step 5a on the first protocol) Add 24 or 48µl of the cocktail to the center of the Tini or Mini Spin Column and close the cap. Centrifuge for 1 minute at 200xg or spin for 30 second pulse at full speed. Reload the flow-through on the center of the column and incubate at 25-37^0C for 15 minutes. This is to ensure that the entire DNase I solution passes through the column. Repeat the step if needed.
2. Add 120µl WASH 2 solution to the column and incubate for additional 5 minutes and then centrifuge ≥12,000 x *g* for 30 seconds. Discard the flow-through.
3. Add 400µl WASH 2 solution to wash the column one more time.
4. Elute the RNA with 10-40ul of RNase-free water.

Storage:

- The RNase-Free DNase Set is shipped at room temperature and should be stored at 2–8ºC immediately upon receipt, which will be stable for at least 9 months.
- Aliquot the stock solution and keep at -80*C for up to 1-2 years. Avoid freeze thaw!

Option 2: Recover small RNA from flow-though **(includes miRNA, 5S rRNA, and tRNA)**

This protocol is designed to recover small RNA by using Tini spin column (EZC107, not included in this kit)

1. Add 0.64 volumes of 100% ethanol to the flow-through from step 4 (first protocol).
2. Apply to the RNA Tini spin column (EZC107).
3. Add 180 ul of WASH 2 to the Tini Spin Column, spin at 14,000xg for 1 min. Discard the flow-through and spin once more to remove residue of RPE solution.
4. Add 180ul of 80% ethanol and wash the column, spin at 14,000xg for 1 min. Discard the flow-through and spin once more to remove residue of ethanol.
5. Transfer the Tini spin columne to a clean RNase-free 1.65ml micro-centrifuge tube and add 5-10 µl of RNase-free water to the center of the column; incubate at room temperature for 2 minutes. Spin to elute the RNA at full speed for 1 minute. RNA is ready for use or kept at -80^0C.

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