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Mammalian RNA Isolation Kit

(Cat# K2065-50, 50 assays, Store at RT)

I. Introduction:

BioVision's Mammalian RNA Isolation Kit allows the quick and simple extraction of RNA from mammalian cells and tissues. Samples are first lysed and homogenized in a specially designed lysis buffer, which inhibits the activity of RNases. After the addition of ethanol, the sample is applied to the RNA column to bind RNA. After 2 wash steps, purified RNA is eluted with RNase-free water. Purified RNA can be used for downstream applications such as RT-PCR, qPCR, etc. Additionally, this kit can be used for clean-up of RNA and RNA enzymatic reactions. The kit enriches for RNA > 200 bp as smaller RNAs do not bind to the column efficiently.

II. Application:

- RNA purification from mammalian cells and tissues or clean-up of RNA enzymatic reactions.

III. Key Features:

- RNA purification from mammalian cells and tissues.
- Clean-up of RNA enzymatic reactions.

IV. Sample Type:

- Up to $1x10^7$ (cultured cells) or 20 mg (tissues)

V. Kit Contents (Mammalian RNA Isolation Kit):

Components	K2065-50 (50 assays)	Cap Code	Part Number
RNA-Lysis Buffer	45 ml	Amber/WM	K2065-50-1
RW1 Buffer	40 ml	NM	K2065-50-2
RW2 Buffer	11.25 ml	NM	K2065-50-3
RNase-free Water	5 ml	NM	K2065-50-4
RNA Columns	50	--	K2065-50-5

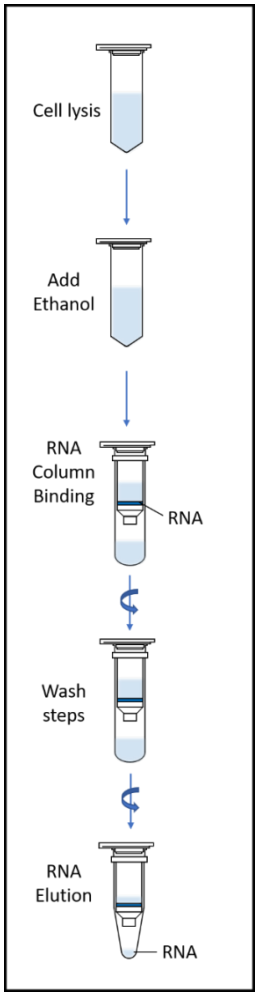
VI. User Supplied Reagents and Equipment:

- 96-100% Ethanol; 70% Ethanol
- β-mercaptoethanol (14.3 M)
- Barrier pipette tips
- 1.5 ml and 2 ml RNase-free microcentrifuge tubes
- Benchtop centrifuge
- RNase-free syringe with 25 gauge needle and/or Dounce Homogenizer (BioVision Cat# 1998)
- On-Column DNase Digestion Kit (BioVision Cat# K2066)

VII. Storage Conditions and Reagent Preparation

Store all the reagents at room temperature (RT) for up to a minimum of 1 year, protected from light.

- **RW1 Buffer: Add 10 ml of (96-100%) Ethanol to RW1 Buffer bottle before use.** Mix well.
- **RW2 Buffer: Add 45 ml of (96-100%) Ethanol to RW2 Buffer bottle before use.** Mix well.
- **RNA-Lysis Buffer:** Determine the amount of RNA-Lysis Buffer required and add 10 µl of β-mercaptoethanol (β-ME) per 1 ml of RNA-Lysis Buffer for immediate use. Prepare fresh. **Notes:** β-ME is recommended for RNase rich cell lines. We recommend using 350 µl RNA-Lysis Buffer for between 1x and $5x10^6$ cells and 600 µl RNA-Lysis Buffer for between 5x and $10x10^6$ cells.



VIII. RNA Purification Protocol:

A. <u>Total RNA Purification Protocol:</u>

1. Sample Lysis:

- **Suspension cells:** Collect cells by centrifuging at 300 x *g* and RT for 5 min. Remove the supernatant completely by aspiration and add RNA-Lysis Buffer.
- **Adherent cells:** Aspirate the media completely with a Pasteur pipet and add RNA-Lysis Buffer to the flask. Scrape cells and move the suspension to a microcentrifuge tube.
  a) Lyse cells by pipetting up and down and passing the sample through a 25 g needle at least 5-10 times.
  b) Ensure minimal sample loss and adjust the volume using RNA-Lysis Buffer, if needed. Entire lysate can be used in step 2.
  Notes: i) Growth media must be removed completely. Residual media will inhibit cell lysis and affect the RNA yield. ii) Adherent cells can alternatively be treated with enzymes, pelleted and treated like suspension cells.
- **Tissue samples:**
  a) Remove the preservation solution completely and weigh the tissue sample (≤ 20 mg). Add 600 µl RNA-Lysis Buffer.
  b) Homogenize sample(s) by using a Dounce Homogenizer or mortar and pestle or by any other method of choice.
  c) Lyse sample further by pipetting up and down and passing the sample through a 25 g needle at least 5-10 times.
  d) Centrifuge at 13,000 x *g* and RT for 5 min to remove any insoluble material. Clarified lysate (supernatant) should be transferred to a new centrifuge tube and used in step 2. **Notes:** i) Sample should not exceed 20 mg. ii) Ensure minimal sample loss and adjust the volume using RNA-Lysis Buffer, if needed. iii) Fatty tissue lysates should be centrifuged for an additional 5 min at 13,000 x *g* and RT.

2. Add 350 µl 70% Ethanol to the lysate (equivalent to 1 volume) and mix by pipetting or inversion. A precipitate may form but will not interfere with the purification step.

3. Transfer 700 µl of the solution from step 2 to a **RNA column**. Centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow-through.

4. If solution remains, repeat step 3.

5. Add 700 µl RW1 Buffer ***(*add Ethanol before use)*** to the same RNA column and centrifuge at 10,000 *x g* for 30 sec. Discard the flow-through. ***Optional:*** *Perform On-Column DNA digestion* (BioVision Cat# K2066) instead of step 5.
6. Add 500 µl RW2 Buffer ***(*add Ethanol before use)*** to the same RNA column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow-through.
7. Add another 500 µl RW2 Buffer to the RNA column and centrifuge at 10,000 x *g* and RT for 1 min.
8. Place the column into a new 2 ml microcentrifuge tube. Centrifuge the column at max speed for 2 min.
9. Transfer the column to an RNase-free 1.5 ml microcentrifuge tube and add 50-100 µl RNase-free Water to the center of the column. Centrifuge at 10,000 x *g* for 1 min at RT to elute the RNA.
10. Store the RNA solution at -80 °C or -20 °C for short term storage. **RNA should be quantified. Note:** For quantification by spectroscopy, an aliquot of RNA should be diluted with 10 mM Tris-HCl, pH 7.5.

B. RNA Clean-Up Protocol:

1. Add RNase-free Water to the RNA enzymatic reaction to adjust the volume to 100 µl.
2. Add 350 µl of RNA-Lysis Buffer to the diluted enzymatic reaction and mix well.
3. Add 250 µl of Ethanol (96-100%) and mix well.
4. Load 700 µl of the solution onto a RNA Column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow-through. ***Optional:*** *Perform On-Column DNA digestion* (BioVision Cat# K2066).
5. Add 500 µl RW2 Buffer ***(*add Ethanol before use)*** to the RNA column. Centrifuge at 10,000 x *g* for 30 sec. Discard the flow-through.
6. Add another 500 µl RW2 Buffer to the column and centrifuge at 10,000 x *g* at RT for 1 min.
7. **Place the column** into a new 2 ml microcentrifuge tube. Centrifuge at max speed at RT for 1 min.
8. Transfer the column to an RNase-free 1.5 ml centrifuge tube. Add 50-100 µl RNase-free Water to the center of the column. Centrifuge at 10,000 x *g* and RT for 1 min to elute the RNA.
9. Store the RNA solution at -80 °C or -20 °C for short term storage. RNA should be quantified.

IX. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low A_{260}/A_{280} ratio	• Protein contamination. • Guanidine Thiocyanate contamination. • Low RNA yield.	• Perform Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected. Add 2.5 volumes of ethanol and 0.1 M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20 °C. Centrifuge at 10,000 g for 15 min at 4 °C. Resuspend the RNA pellet in RNase free water. • Perform RNA Clean-Up Protocol. • Ensure that the sample is diluted in 10 mM Tris, pH 7.5. Use 10 mM Tris as blank.
Low Yield	• Not enough cells/ insufficient homogenization. • RNA in sample degraded. • The binding capacity of the membrane in the spin column was exceeded. • Ethanol not added to buffer.	• Increase the sample size. • Ensure samples are properly homogenized. • Freeze samples immediately after collection in liquid nitrogen and store at -70 °C. Prepare fresh cultures. Using too much tissue sample will result in poor yield of RNA.
Genomic DNA contamination	• Too much total RNA sample was used in RT-PCR. • The sample may contain too much genomic DNA. • Too little starting material	• Reduce the amount of RNA to 50-100 ng for RT-PCR. • Reduce the amount of starting tissue. Most tissues will not show a large genomic DNA contamination problem at 20 mg or less per prep. • The RNA column preferentially binds to RNA. But if RNA concentration is very low more DNA will bind. • Perform On-Column DNA digestion or DNA digestion followed by RNA Clean-Up.

IX. Functional Test Data:

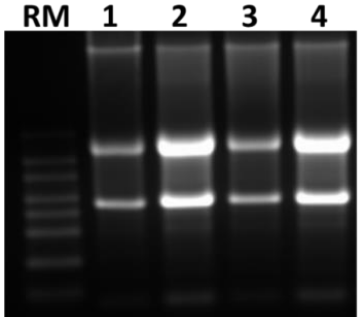


Figure 1: RNA was purified from 5 x10^6 (Lanes 1 & 3) and 1x10^7 (Lanes 2 & 4) Jurkat cells stored in RNAkeep™ Solution (BioVision Cat# M1241) using Mammalian RNA Isolation Kit (BioVision Cat# K2065). 10 µl RNA was loaded per lane and analyzed on a 1% agarose gel. RM is Riboruler marker.

X. Related Products:

EasyRNA™ Blood RNA Mini Kit (K1373)
EasyRNA™ Bacterial RNA Kit (K1351)
On-Column DNase Digestion Kit (K2066)
EasyRNA™ Plant RNA Mini Kit (K1374)
One Prep DNA/RNA Purification Kit (K2058)
EZQuant™ RNA Quantification Kit II (K1480)
Yeast RNA Mini Kit (K1418)
RNAkeep™ Solution (M1241)
Virus DNA/RNA Kit (K1360)

FOR RESEARCH USE ONLY! Not to be used on humans.