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miRNA Extraction Kit

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(Catalog # K1456-50; 50 Preps; Ship at RT and Store at Multiple Temperatures)

I. Introduction:

BioVision's miRNA Extraction Kit is used to purify miRNA from cells and tissues. This kit is based on an improved guanidine thiocyanate-phenol one step method and elution of miRNA using spin columns. In this kit, the ribonucleases are inactivated and the genomic DNA, 18S and 28S RNAs are removed using spin columns. Cellular metabolite and proteins are removed by a series of elution-centrifugation steps. Subsequently, the microRNAs (including miRNA, snRNA, and other RNA less than 200 bp) are eluted from a second spin column in RNase-free water. The purified miRNA is ready for downstream applications including RNA sequencing, microarray, RT-PCR, Northern Blot etc.

II. Applications:

- RNA sequencing, microarray, RT-PCR, Northern Blot

III. Key Features:

- Purify miRNA, snRNA,and other RNA less than 200 bp
- **Highly pure miRNA** for downstream applications
- Genomic DNA is efficiently removed by buffer MRL
- **High quality Spin Columns** with silica membranes

IV. Sample types:

- Cells and tissues (plant, animal, fungi and bacteria)

V. Kit Contents:

Components	K1456-50 (50 Preps)	Part Number
Buffer MRL	55 ml	K1456-50-1
***Buffer RW	15 ml	K1456-50-2
RNase-free water	10 ml	K1456-50-3
****RNase-free water (for 70% Ethanol)	9 ml	K1456-50-4
RNase-free Spin Column RA	50	K1456-50-5
RNase-free Spin Column RB	50	K1456-50-6
Collection Tube	50	K1456-50-7

Buffer RW must be diluted with Ethanol before use. Add 60 ml Ethanol to the Buffer RW bottle before use and vortex adequately. Be sure to close the bottle tightly after each use to avoid Ethanol evaporation.

****Add 9 ml of RNase-free water to 21 ml of 100% Ethanol to prepare 70% Ethanol.***

VI. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- Absolute Ethanol (100%)
- RNase free water
- Chloroform
- 1.5 ml microcentrifuge tubes
- TE Buffer
- Microcentrifuge

VII. Shipping and Storage Conditions:

All kits are shipped at room temperature (RT). All reagents except buffer MRL must be stored at RT. Buffer MRL must be stored at 4°C in the dark. All reagents are stable for up to 12 months when stored properly at RT and 4°C.

VIII. Reagent Preparation and Storage Conditions:

1. Buffer RW must be diluted with ethanol before use. Add 60 ml ethanol to Buffer RW bottle and vortex adequately.
2. Precipitates may form in buffers at low temperatures. Incubate at 37°C briefly until clear, then cool down to 25°C before use.
3. The kit is transported at RT. Buffer MRL can be transported at RT but should be stored at 4°C in the dark upon arrival.
4. Add 9 ml of RNase-free water to 21 ml of 100% ethanol to prepare 70% ethanol.

IX. Assay Protocol:

General Precautions to handle RNA:

1. To prevent RNA degradation, all the centrifugation steps should be done at 4°C, unless otherwise specified.
2. Wear gloves all the time to avoid RNA degradation by RNAses.
3. Whenever possible, use sterile disposable plasticware for handling RNA.
4. Heat glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1 N NaOH, 1 mM EDTA followed by RNase-free water.

Protocol:

1. Homogenization Step:

a. **Tissues: Homogenize** 50-100 mg tissue in 1 ml of **Buffer MRL** until there is no visible tissue. For tissues stored in liquid nitrogen, grind the tissue into a fine powder using a mortar and pestle. Make sure that the volume of the liquid nitrogen sample is not more than 1/10 of the total volume of Buffer MRL.

b. **Adherent cells:** Add **Buffer MRL** (1 ml per 10 cm^2) to the culture plate containing 10^6 cells and completely lyse cells by pipetting. Genomic DNA contamination may occur if sufficient buffer MRL is not used.

c. **Suspension Cells:** Harvest cells in **Buffer MRL** into a microcentrifuge tube by centrifugation (animal, plant, fungus, 5~10×10^6 cells per 1 ml; bacteria 1×10^7 cells per 1 ml).

The samples homogenized in Buffer MRL (without Chloroform) can be stored at -70°C.

2. Mix vigorously and incubate the mixture for 5 min to lyse ribosomal particles completely.
3. **Optional:** Centrifuge the mixture at 12,000 g for 10 min at 4°C. Remove upper, aqueous phase to an RNase-free tube. Alternative protocols are required when the sample is rich in proteins, fats, amylase and other extracellular substances (eg, muscle) and plant tubers.
4. Add 200 µl of **Chloroform** to 1 ml of Buffer MRL mixture. Mix vigorously for 15 sec and incubate for 3 min at RT.
5. Centrifuge the mixture at 12,000 g for 10 min at 4°C. Transfer the upper, aqueous phase that contains RNA to an RNase-free tube.
6. Precipitate the aqueous phase by the addition of an equal volume (500 µl) of **70% Ethanol** (make sure ethanol is added), mix gently. You may notice a flocculated precipitate.
7. Add the solution and the flocculated precipitate from step 6 into an **RNase-free Spin Column RA** placed in a **Collection Tube**.
8. Centrifuge at 10,000 g for 45 sec, collect the flow-through containing microRNA. Check the volume of flow-through exactly. Add **70% Ethanol** (2/3 volume of flow-through) and mix gently.
9. Add this mixture to an **RNase-free Spin-column RB**. Centrifuge at 10,000 g for 30 sec at 4°C. If the volume is more than 700 µl, repeat the centrifugation process in the **same Spin Column**, and discard the flow-through.

If you want to separate the macro RNA (18S and 28S), use the Spin Column RA from step 8 and proceed with the following steps:

10. Add 700 µl of **Buffer RW** with Ethanol to the **Spin Column**, centrifuge at 12,000 g for 60 sec, and discard the flow through.
11. Add 500 µl of **Buffer RW** with Ethanol, centrifuge at 12,000 g for 60 sec. Discard the flow through.
12. Place the **Spin Column RB** back in the tube, centrifuge at 12,000 g for 2 min. Discard all the Ethanol as it will inhibit downstream reactions.
13. Place the **Spin-Column RB** in a new **Collection Tube**. Add 60-80 µl **RNase-free water** (warmed at 65-70°C). Incubate for 2 min at RT, centrifuge at 12,000 g for 1 min. Store the **eluted microRNA** at -20°C or -80°C.
14. The integrity of the purified RNA may be determined by denaturing agarose gel electrophoresis. The ratio of ~5 kB（28S）to ~2 kB（18S) ribosomal RNA should be approximately 2:1 by ethidium bromide staining. The purity of miRNA can be determined by OD_{260}/OD_{280}. Alternatively miRNA can be eluted in **TE buffer** (not provided).

X. Related Products:

Product Name	Cat. No.	Size
EasyRNA™ Blood RNA Mini Kit	K1373	50, 250 Preps
Yeast RNA Mini Kit	K1418	50, 250 Preps
EasyRNA™ Cell/Tissue RNA Mini Kit	K1337	50, 250 Preps
EasyRNA™ Plant RNA Mini Kit	K1374	50, 250 Preps
EasyRNA™ Fungal RNA Kit	K1419	50, 250 Preps
EasyRNA™ Bacterial RNA Kit	K1351	50, 250 Preps

XI. General Troubleshooting Guide:

Problems	Causes	Solutions
Low yield	Tissue was not homogenized thoroughly.	Grind fresh tissue in Buffer MRL using a mortar and pestle. For tissues stored in liquid nitrogen, grind the tissue into a fine powder after adding buffer MRL. Completely lyse the cells by pipetting or vortexing.
	RNA was degraded.	Take fresh samples for isolation of miRNA.
	The sample was low in RNA.	RNA percentage varies with tissues and cells. Some samples need more homogenization.
	Sample was more than the binding capacity of the silica membrane.	Use multiple spin-columns RA for the same sample.
	Ethanol was not added to Buffer RW.	Please add ethanol to Buffer RW.
OD_{260}/OD_{280} <1.6	Dissolving RNA in water causes higher OD_{280} because of lower ion intensity and pH.	Dissolve RNA in TE buffer.
Genomic DNA contamination	Sample contains some chemical solvents (such as ethanol, DMSO alkaline solutions etc).	Avoid these chemicals.
	May have taken the middle phase in Step 5.	Do not take the middle phase in Step 5. Take the upper aqueous phase.
Integrity of RNA is not good	Glassware and plasticware were not treated before use.	Treat non-disposable glassware and plasticware before use to ensure that everything is RNase-free.
	RNA may have been degraded during sample preparation.	To avoid RNA degradation, homogenize samples in Buffer MRL or freeze samples in liquid nitrogen and store at -70°C if they cannot be immediately processed.
	RNA was not stored properly	Store at -70°C.
	RNA was degraded during processing.	Work quickly during sample preparation and maintain the sample lysate at 4°C.
Downstream RT-PCR was not successful	When taking the spin-column out, it came into contact with ethanol.	Take the spin-column out carefully, and leave aside for a few minutes for ethanol evaporation. Ethanol inhibits RT-PCR.