

Hieff™ Cell/Tissue miRNA Kit

Product description

This Kit is suitable for the extraction of total RNA, miRNA, and various small RNAs from all samples that can be extracted using the TRIzol method, including various animal and plant tissues, bacterial tissues, and cultured cells. The silica-based material used in the centrifuge adsorption column is a proprietary new material from our company, which, combined with our unique lysis buffer formulation, can maximize the recovery of high-purity RNA and miRNA. The extracted RNA has high purity, stable and reliable quality, is suitable for RT-qPCR and other downstream applications.

Specifications

Cat.No.	19331ES08 / 19331ES50
Size	5 T / 50 T

Components

Category	Components No.	Name	19331ES08	19331ES50
Part I	19331-A	Lysis Buffer(LB Buffer M1)	5 mL	50 mL
Part II	19331-B	Hieff™ RNA Adsorption Column M1	5 pieces	50 pieces
	19331-C	miRNA Column M1	5 pieces	50 pieces
	19331-D	2 mL Collection Tube M1	10 pieces	100 pieces
	19331-E	PL Buffer M1*	1.2 mL	12 mL
	19331-F	Wash Buffer M1*	1.3 mL	13 mL
	19331-G	RNase-free H ₂ O	1 mL	5 mL

Storage

Part I components should be stored at 4~8°C for 2 years.

Part II components should be stored at room temperature for 2 years.

Instructions

Preparation before the experiment

1. Required equipment and reagents: tabletop centrifuge, shaker, water bath or metal bath, chloroform, absolute ethanol, RNase-free centrifuge tubes, etc.
2. Unless otherwise specified, all centrifugation steps are performed at room temperature using a conventional tabletop centrifuge capable of reaching 12,000 rpm.
3. Before the first use, add the specified volume of absolute ethanol to the **PL Buffer M1*** and **Wash Buffer M1*** bottles according to the table below, mix thoroughly before use, and label it properly.

Number	Component name	5 T specification amount of absolute ethanol to be added	50 T specification amount of absolute ethanol to be added
19331-E	PL Buffer M1*	2.8 mL	28 mL
19331-F	Wash Buffer M1*	5.2 mL	52 mL

I. Sample Preprocessing:

1. Sample Homogenization

A. For adherent cells: Discard the culture medium, and directly add 1 mL **LB Buffer M1** to a 3.5 cm diameter culture plate, covering and repeatedly pipetting to lyse the cells.

[Note]:

1. Determine the required amount of **LB Buffer M1** based on the area of the culture plate rather than the number of cells (add 1 mL per 10 cm²).
 2. Insufficient addition of **LB Buffer M1** can lead to DNA contamination.
 3. Adherent cells often cannot be completely detached from the culture flask (dish), which does not mean that lysis is incomplete. At this point, the cell membrane has actually completely ruptured, releasing RNA, and you can proceed with the subsequent experiments.
- B. Suspended cells: Centrifuge to collect the cell pellet, adding 1 mL **LB Buffer M1** for every $5 \times 10^6 \sim 1 \times 10^7$ cells, and repeatedly pipette to mix.

[Note]: Before adding **LB Buffer M1**, avoid washing the cells, as this may increase the likelihood of mRNA degradation.

C. Plant and animal tissues: Take 50~100 mg of tissue and grind thoroughly in liquid nitrogen, then add 1 mL **LB Buffer M1** to homogenize.

[Note]: 1. The sample volume should not exceed 10% of the volume of **LB Buffer M1** added.

2. After vigorously shaking the homogenized sample, incubate at room temperature for 5 min to allow complete dissociation of the nucleoprotein.
3. Add 0.2 mL of **chloroform (self-provided)** to every 1 mL of **LB Buffer M1**. Tightly close the sample tube lid, vigorously shake for 15 sec, and then incubate at room temperature for 3 min.
4. Centrifuge at 12,000 rpm for 10 min, the sample will separate into three layers: the lower organic phase, the middle layer, and the upper colorless aqueous phase, with RNA present in the aqueous phase. The volume of the aqueous phase is approximately 50%-60% of the added **LB Buffer M1** volume, carefully transfer the upper aqueous phase to a new tube (do not touch the middle layer), and record the volume of the aqueous phase (approximately 600 μ L).

II. Total RNA Extraction: (Extraction of total RNA containing miRNA) (Recommended)

1. Insert **HieffTM RNA Column M1** into **2 mL Collection Tube M1** and set aside.
2. Add 1.5 times the volume of the upper aqueous phase of room temperature **absolute ethanol (self-provided)** to upper aqueous phase, and immediately mix by pipetting.

[Note]: The appearance of precipitate is a normal phenomenon.

3. Add the above mixture to **HieffTM RNA Column M1**, centrifuge at 12,000 rpm for 1 min, and discard the waste liquid.

[Note]: The maximum volume of the mixture that can be added at one time is 650 μ L, and it can be centrifuged in multiple batches.

4. Add 700 μ L **PL Buffer M1*** centrifuge at 12,000 rpm for 30 sec, discard the waste liquid.

[Note]: Ensure that **PL Buffer M1*** has been added with absolute ethanol.

5. Place **HieffTM RNA Column M1** back into the collection tube, add 500 μ L **Wash Buffer M1***, centrifuge at 12,000 rpm at room temperature for 30 sec, discard the waste liquid.

[Note]: Ensure that **Wash Buffer M1*** has been added with absolute ethanol.

6. Repeat step 5.

7. Place **HieffTM RNA Column M1** back into the collection tube, centrifuge the empty column at 12,000 rpm for 2 min at room temperature to remove residual **Wash Buffer M1***.

8. Place **HieffTM RNA Column M1** into a new 1.5 mL RNase-free centrifuge tube, add 30-50 μ L RNase-free H₂O to the center of **HieffTM RNA Column M1**, and let it sit at room temperature for 2 min. Then centrifuge at 12,000 rpm for 1 min. Collect the filtrate, which is the RNA solution.

[Note]: The recovery yield can be improved by the following methods: ① Preheat RNase-free H₂O to 65°C; ② Pass the RNA filtrate through the column again, let it sit at room temperature for 2 min, and then elute.

9. The RNA solution can be stored at -80°C for long-term preservation.

III. microRNA enrichment: (only extracting microRNA, excluding other RNAs >200 nt)

1. Insert **HieffTM RNA Column M1** and **miRNA Column M1** into **2 mL Collection Tube M1** respectively, and set aside.

2. Add 0.5 times the volume of **absolute ethanol (self-provided)** to upper aqueous phase and mix thoroughly by pipetting.

[Note]: Absolute ethanol must be stored at room temperature.

3. Add the above mixture to **HieffTM RNA Column M1** and centrifuge at 12,000 rpm for 1 min, **collect the filtrate**. And transfer the filtrate to a new RNase-free centrifuge tube.

[Note]: The maximum volume of the mixture that can be added at one time is 650 μ L, and it can be centrifuged in multiple batches.

[Note]: miRNA is in the filtrate, while the total RNA is retained on the adsorption column to remove miRNA.

4. Calculate the volume of the filtrate, add 0.65 times the volume of absolute ethanol at room temperature, and mix thoroughly by pipetting.

5. Add the above mixture to **miRNA Column M1**, centrifuge at 12,000 rpm for 1 min, and discard the filtrate.

[Note]: The maximum volume of the mixture that can be added at one time is 650 μ L, and it can be centrifuged in multiple batches.

[Note]: After centrifugation, if there is still liquid residue on the membrane, the centrifugation time can be extended to 5 min.

6. Follow the **Total RNA (including miRNA) extraction method** steps 4~9 to wash and obtain miRNA.

[Note] : In this operation, the centrifuge columns used in steps 4~9 should be **miRNA Column M1**.

Notes

1. Avoid prolonged exposure of reagents to air to prevent volatilization, oxidation, and pH changes. All solutions should be promptly capped after use.
2. **Wash Buffer M1*** After adding ethanol, it is prone to precipitation, but does not affect usage. When using, simply aspirate the supernatant.
3. **LB Buffer M1** and **PL Buffer M1*** contain irritants. For your safety and health, please wear a lab coat and disposable gloves while handling.
4. This product is for research use only!