

Hieff™ Serum/Plasma miRNA Kit

Product description

This Kit is suitable for the extraction of total RNA and miRNA from samples such as plasma, serum, and lymphatic fluid. The silica-based material used in the centrifuge adsorption column is a proprietary new material from our company, combined with our unique lysis buffer formulation, which maximizes the recovery of high-purity RNA and miRNA. The extracted RNA has high purity and stable, reliable quality, making it suitable for downstream applications such as RT-qPCR.

Specifications

Cat.No.	19332ES08 / 19332ES50
Size	5 T /50 T

Components

Category	Components No.	Name	19332ES08	19332ES50
Part I	19332-A	Lysis Buffer(LB Buffer S3)	5 mL	50 mL
Part II	19332-B	Hieff™ RNA Adsorption Column S3	5 pieces	50 pieces
	19332-C	miRNA Column S3	5 pieces	50 pieces
	19332-D	2 mL Collection Tube S3	10 pieces	100 pieces
	19332-E	PL Buffer S3*	1.2 mL	12 mL
	19332-F	Wash Buffer S3*	1.3 mL	13 mL
	19332-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 Use

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 first use, add the specified volume of absolute ethanol to the PL Buffer S3* and Wash Buffer S3* bottles as per the table below, mix thoroughly, and label accordingly.**

Number	Component Name	5 T specification amount of absolute ethanol to be added	50 T specification amount of absolute ethanol to be added
19332-E	PL Buffer S3*	2.8 mL	28 mL
19332-F	Wash Buffer S3*	5.2 mL	52 mL

Operating Procedure

I. Sample Preprocessing:

1. Transfer 250 μ L of serum or plasma liquid sample to a 1.5 mL RNase-free centrifuge tube.

[Note]: If less than 250 μ L, supplement with PBS or saline.

2. Add 750 μ L **LB Buffer S3**, repeatedly pipette up and down, and vigorously vortex to mix.

3. Let it stand at room temperature (22-30°C) for 5 min.

4. Add 200 μ L of **chloroform (provided by yourself)**, and shake vigorously for 15 sec to mix.

5. Incubate at room temperature for 2 min.

6. Centrifuge at 12,000 rpm for 10 min at 4°C to separate the sample. Transfer the upper aqueous phase to a 1.5 mL RNase-free centrifuge tube.

[Note]: The sample will separate into three layers; the bottom layer is the organic phase, the middle layer, and the upper colorless aqueous phase, where RNA is present in the upper aqueous phase.

[Note]: The volume of the upper phase is approximately 70% of the total volume of LB Buffer S3 added. If 750 μ L of LB Buffer S3 is added, the upper aqueous phase will be approximately 525 μ L. It is recommended to aspirate 500 μ L to avoid contaminating with the middle layer, which may contain DNA.

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

1. Insert **HieffTM RNA Column S3** into **2 mL Collection Tube S3** and set aside.

2. Add 1.5 times the volume of absolute ethanol (self-provided) to upper aqueous phase and mix thoroughly.

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

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

3. Add the above mixture to **HieffTM RNA Column S3** and centrifuge at 12,000 rpm for 1 min, discarding the waste liquid.

[Note]: The maximum volume of the mixture that can be added at one time is 650 μ L; multiple centrifugations can be performed.

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

4. Add 700 μ L **PL Buffer S3***, centrifuge at 12,000 rpm for 30 sec, discard the waste liquid.

[Note]: Ensure that **PL Buffer S3*** has been added with absolute ethanol.

5. Place **HieffTM RNA Column S3** back into the collection tube, add 500 μ L **Wash Buffer S3***, centrifuge at 12,000 rpm at room temperature for 30 sec, discard the waste liquid.

[Note]: Ensure that **Wash Buffer S3*** has been added with absolute ethanol.

6. Repeat step 5.

7. Place **HieffTM RNA Column S3** back into the collection tube, centrifuge the empty column at 12,000 rpm at room temperature for 2 min to remove residual **Wash Buffer S3***.

8. Place **Hieff™ RNA Column S3** into a new 1.5 mL RNase-free centrifuge tube, add 30–50 µL RNase-free H₂O to the center of **Hieff™ RNA Column S3**, and let it sit at room temperature for 2 minutes. Then centrifuge at 12,000 rpm for 1 minute. 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 Reapply the RNA filtrate to the column, let it sit at room temperature for 2 minutes, 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 **Hieff™ RNA Column S3** and **miRNA Column S3** into 2 mL collection tube separately, and set aside.

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

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

3. Add the above mixture to **Hieff™ RNA Column S3**, centrifuge at 12,000 rpm for 1 min, **collect the filtrate**. 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 µL; multiple centrifugations can be performed.

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

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

5. Add the above mixture to **miRNA Column S3**, centrifuge at 12,000 rpm for 1 min, **discard** the filtrate.

[Note]: The maximum volume of the mixture that can be added at one time is 650 µL; multiple centrifugations can be performed.

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

6. Follow the **total RNA (including miRNA) extraction method** steps 4~9 for washing to obtain miRNA.

[Note]: In this operation, the centrifuge columns used in steps 4~9 should be **miRNA Column S3**.

Notes

1. Avoid prolonged exposure of reagents to air to prevent volatilization, oxidation, and pH changes. Each solution should be tightly capped immediately after use.

2. After adding ethanol to wash Buffer W*, it is prone to precipitation, but this does not affect its use. When using, simply aspirate the supernatant.

3. **LB Buffer S3** and **PL Buffer S3*** contain irritants. For your safety and health, please wear a lab coat and disposable gloves while handling.

4. This product is for research purposes only!