GRS microRNA Purification Kit

#GK11.0050 (50 preps) | GK11s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 200µl of fresh blood, up to 1x10^6 cultured cells, up to 100mg of tissue, up to 25 mg of formalin-fixed paraffin-embedded tissue (FFPE)
Expected Yield : miRNA (and other small cellular RNAs)
Format : spin column
Operation Time : 30 minutes
Elution Volume : 50µl

Product: Standard protocols for the isolation of total RNA or mRNA are not optimized for the purification of small RNA molecules, resulting in the loss of considerable amounts of micro RNAs (miRNAs) and other small cellular RNAs. Moreover, for accurate analysis of expression of miRNAs by qPCR or by microarray analysis requires the removal of the larger RNA molecules. The GRS microRNA Kit provides an efficient and fast method for the purification of high-quality miRNAs (and other small cellular RNAs), with minimal contamination from large RNA molecules and genomic DNA, from a variety of samples, including fresh blood, cultured cells, tissue, and FFPE.

The GRS microRNA Kit uses chaotropic salts and various ethanol concentrations to allow selective binding of RNA of specific sizes to the glass fiber matrix of the spin column[1]. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified RNA is subsequently eluted with a specific RNase-Free Elution Buffer. The entire procedure can be completed within 30 minutes.

Caution: Buffers contain chaotropic salt which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

In order to prevent RNase contamination, one should use disposable plasticware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-free and used only for RNA procedures. During handling, gloves should be worn at all times.

References:

1. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

Kit Contents	(50 preps)	(4 preps)
Lysis Buffer	12 ml	1 ml
miRNA Buffer	1.5 ml	1.5 ml
Wash Buffer*	12.5 ml	250 µl
miRNA Elution Buffer	6 ml	1 ml
RNA mini spin column	100	8
2-ml collection tube	100	8
1.5-ml microtube (DNase/RNase free)	200	-
Micropestle	50	4

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets (and tips) (RNase-Free)
Vortex
Water bath or Thermoblock
Chloroform
Phenol (ddH_2O saturated)
Xylene for "FFPE samples"
Red Blood Cell Lysis Buffer for "fresh blood samples" (ref: GKRBCLB.0100)

* Add Ethanol (96%-100%) [not included] to Wash Buffer, as indicated on the bottle/tube, prior to initial use. After Ethanol has been added, mark the bottle/tube to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.

Storage: All components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37ºC followed by cooling to 25ºC.

PRIOR TO USE:

PRE-PREPARATION OF FRESH BLOOD SAMPLES

1) Transfer up to 200µl of fresh blood in a 1.5-ml microcentrifuge tube (RNase-Free) and add 3 volumes of Red Blood Cell Lysis Buffer (not provided, can be purchased separately) [e.g., add 600µl of Red Blood Cell Lysis Buffer to 200µl of Blood].

2) Incubate for 5 minutes at room temperature and centrifuge at 3,000g for 5 minutes. Remove the supernatant completely and proceed with the lysis of the leukocyte pellet (step 1d at page 3)

PRE-PREPARATION OF PARAFFIN-EMBEDDED TISSUE

1) Slice small sections, up to 25mg, from blocks of paraffin-embedded tissue and transfer to a 1.5-ml microcentrifuge tube (RNase-Free).

2) Add 1ml of Xylene. Vortex vigorously and incubate at room temperature for 10 minutes. During incubation, vortex regularly.

3) Centrifuge at 14,000g-16,000g for 3 minutes. Discard the supernatant.

4) Add 1ml of absolute ethanol to wash the pellet. Mix by inverting. Repeat steps 3 and 4.

5) Centrifuge at 14,000g-16,000g for 3 minutes. Remove the supernatant and incubate the tube with open lid at 37ºC for 10-20 minutes to evaporate any residual ethanol. Proceed with the lysis of the FFPE pellet (step 1b at page 3).

PROTOCOL FOR miRNA PURIFICATION

1)
   a) Add 200µl of Lysis Buffer into a 1.5-ml microcentrifuge tube (RNase-Free) containing up to 100mg of tissue and use a RNase-free micropestle to grind the tissue. Incubate for 10 minutes at room temperature.
   b) Or add 200µl of Lysis Buffer into the 1.5-ml microcentrifuge tube (RNase-Free) containing FFPE pellet (see page 2) and use a RNase-free micropestle to grind the tissue. Incubate for 10 minutes at room temperature.
   c) Or add 200µl of Lysis Buffer into a 1.5-ml microcentrifuge tube (RNase-Free) containing cell pellet (up to $1x10^6$ cultured cells) and vortex vigorously until the pellet is completely resuspended. Incubate for 10 minutes at room temperature.
   d) Or add 200µl of Lysis Buffer into the 1.5-ml microcentrifuge tube (RNase-Free) containing leukocyte pellet (see page 2) and resuspend the leukocyte pellet by pipetting. Incubate for 10 minutes at room temperature.

2) Add 20µl of miRNA Buffer, 180µl of ddH_2O saturated phenol (not provided), and 40µl of chloroform (not provided) to the lysate and mix well by vortexing for 2 minutes [At this time, pre-heat the miRNA Elution Buffer to be used in step 8].

3) Centrifuge at 14,000g-16,000g for 3 minutes. Transfer the upper phase to a new 1.5-ml microcentrifuge tube (RNase-free) and add a 35% volume of absolute ethanol. Mix well by shaking vigorously [e.g., Add 108µl of absolute ethanol to 200µl since (108/(200+108)=0.35)].

4) Place an RNA mini spin column in a 2-ml collection tube and transfer the ethanol-added mixture to the center of the column. Incubate at room temperature for 1 minute and centrifuge at 14,000g-16,000g for 30 seconds. Large RNA molecules will bind, whereas small RNA molecules will not.

5) Transfer the filtrate to a new 1.5-ml microcentrifuge tube (RNase-free) and add a 70% volume of absolute ethanol. Mix well by shaking vigorously [e.g., Add 700µl of absolute ethanol to 300µl of filtrate since (700/(300+700)=0.70)].

6) Place a new RNA mini spin column in a 2-ml collection tube and transfer the ethanol-added mixture to the center of the column. Incubate at room temperature for 1 minute and centrifuge at 14,000g-16,000g for 30 seconds

7) Add 200µl of Wash Buffer*, incubate at room temperature for 1 minute, and centrifuge at 14,000g-16,000g for 1 minute (*Ensure ethanol was added first time prior to use this kit).

8) Discard the collection tube and place the spin column in a new 1.5-ml microcentrifuge tube (RNase-free). Pipet 50µl of miRNA Elution Buffer (preheated to 65ºC) directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3 minutes.

9) Centrifuge at 14,000g-16,000g for 3 minutes to elute purified microRNA.

TROUBLESHOOTING

1. **Low Concentration**
    i. If desired, miRNA can be further concentrated by standard ethanol precipitation, followed by re-dissolving in a small volume of RNase-free water
2. **Low Quality**
    i. Quality can be checked by using 10µl to run on a polyacrlyamide gel. The majority of visible RNA should be smaller than 100 nt, with the major bands corresponding to tRNAs (5S and 5.8S might also be visible). Note that tRNA and small rRNA bands should be clear and distinct, whereas miRNAs (21-22nt) are typically not detectable on the gel.