GRS microRNA Purification Kit

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

PROTOCOL FOR miRNA PURIFICATION from samples stored in tripleXtractor

1) Transfer 500µl of RNA sample in tripleXtractor. Add add 20µl of miRNA Buffer and 100µl of acid phenol-chloroform (not provided), and incubate at room temperature for 10 minutes.

2) Centrifuge at 14,000g-16,000g for 15 minutes with cooling (+6ºC).

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