Promega

Product Application

Automated Purification of Total RNA from Exosomes

Purify total RNA, including miRNA, from plasma or cell culture exosomes using the Maxwell® RSC miRNA Plasma and Serum Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)

Analyses: RT-qPCR

Sample Type: Isolated exosomes

Input: ≤ 200µl in water or non-PBS buffer (e.g., TBS or TE)
≤ 75µl in PBS
≤ 230µl in Lysis Buffer C

Materials Required:

- Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block (set to 37°C)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM546, available at:
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Protocol: *The following protocol is taken directly from the Maxwell® RSC miRNA Plasma and Serum Kit Technical Manual (TM546).*

Note: This kit does not isolate exosomes.

1. Up to 200µl of exosome sample in water or non-PBS buffer can be used. For optimal results, do not use more than 75µl of exosomes resuspended in PBS. For exosome samples less than 200µl, add Nuclease-Free Water to the sample to bring the total volume to approximately 200µl. For exosomes suspended in Lysis Buffer C, see Section 4.A of the Technical Manual.
2. Add 80µl of Proteinase K Solution and 230µl of Lysis Buffer C to the exosome sample. Mix by vortexing for 5 seconds.
3. Incubate at 37°C for 15 minutes. During this time, prepare the Maxwell® RSC Cartridges as described in Section 5.C of the Technical Manual.
4. Transfer all the lysate to well #1 of the Maxwell® RSC Cartridge.
5. Add 10µl of blue DNase I Solution (Section 4.A) to well #4 of the Maxwell® RSC Cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.
6. Proceed to Section 6 of the Technical Manual for instructions on loading samples onto the instrument. Begin the automated purification run.

Results: Conditioned cell media was collected from cells in culture and exosomes were isolated by ultracentrifugation. Isolated exosomes were resuspended in 50µl of PBS per 1ml of cell culture medium input, then diluted with 150µl of Nuclease-Free Water and purified in triplicate using the Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680). miRNA targets were detected by 2-step RT-qPCR using TaqMan™ MicroRNA assays (Applied Biosystems).

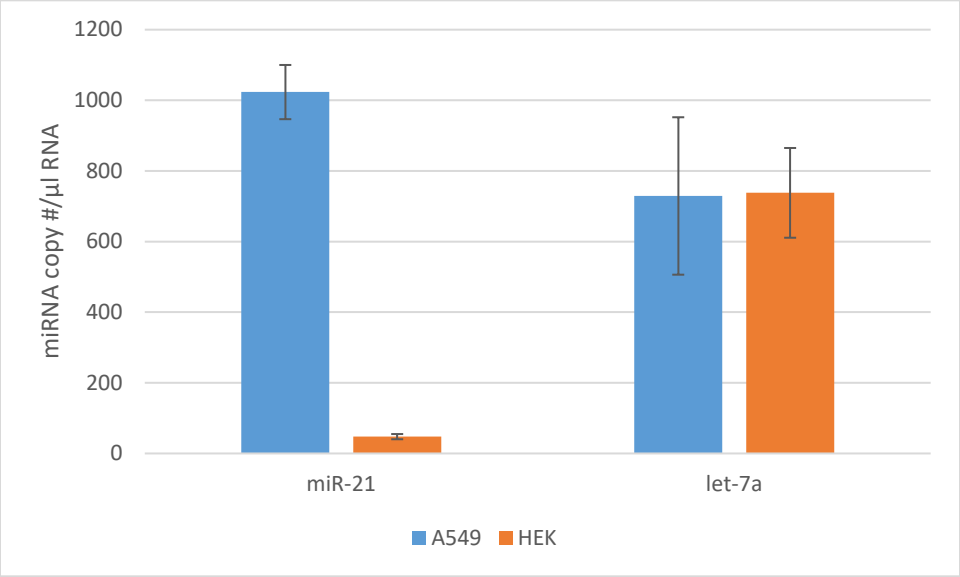

Figure 1. Amplification of let-7a and miR-21 from total RNA purified from conditioned cell culture medium using the Maxwell® RSC miRNA Plasma and Serum Kit on the Maxwell® RSC Instrument. Exosomes from HEK293 and A549 conditioned cell culture media were prepared as described above and total RNA purified in triplicate according to the Technical Manual. 5µl of total RNA was used as the template for multiplexed reverse transcription with the TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems, Cat.# 4366597) using TaqMan™ 20X RT primers for let-7a (Cat.# 440887 Assay ID: RT000377) and miR-21 (Cat.# 440888 Assay ID: RT000397). 3µl of cDNA was used as a template in duplicate 20µl qPCR reactions with either let-7a or miR-21 primer mix and GoTaq® Probe qPCR Master Mix (Cat.# A6101) on an Applied Biosystems™ 7500 Fast Real-Time PCR System. miRNA concentration was calculated relative to a serial dilution of standards included in the qPCR plate.