

EXOBX® Exosomes microRNA Extraction Kit (Magnetic Beads-Based)

Cat. no. R6307

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Catalog Number	Reagent In Kit	Size	Storage
R6307	Lysis Buffer for microRNA	100 mL	RT
	Wash Buffer C	30 mL	RT
	Wash Buffer D	9 mL	RT
	Elution Buffer R	10 mL	RT
	Magnetic Beads	1.5 ml	4 °C
	DNA Clear Column	30 sets	RT
	Manual	1	

1. Transportation and Storage

- 1) This product is transported at room temperature, and valid for 2 years.
- 2) Magnetic beads are stored at 4 °C, and the other reagents in the kit are stored at room temperature.

2. Important Product Information

- 1) The magnetic beads should be stored at 4 °C and are forbidden to freeze, or they become invalid.
- 2) The whole experimental process should be carried out on ice.
- 3) Please use RNase-free appliances and consumables, such as microcentrifuge tubes, tips and pipette.
- 4) Repeat freezing and thawing for exosomes may promote microRNA degradation, please use fresh exosomes or exosomes stored at -80 °C to isolate microRNA.
- 5) DNA Clear Column can effectively remove DNA from exosomes microRNA, which significantly improve the purity of microRNA.
- 6) Please wear lab clothes, gloves and safety glasses during the experiment.

3. Product Introduction

The kit is specifically designed for isolating microRNA from exosomes based on the unique microRNA isolation reagents and magnetic beads, which could efficiently isolated microRNA from exosomes with high purity. Therefore, the isolated exosome microRNA could be used for various subsequent experiments, including real-time fluorescence quantitative PCR (QPCR), sequencing and other experiments.

4. Additional Materials Required

- Ethanol
- Isopropyl alcohol
- Variable-speed bench-top microcentrifuge (refrigerated)
- RNase-free tubes and tips.
- Vortex Mixer
- Magnetic Racker
- Rotary Mixer

5. Experimental Procedure

- 1) Reagents Preparation. Add 21 mL ethanol to Wash Buffer D and mix thoroughly.
- 2) Add 3 ml Lysis Buffer for microRNA into 1 ml exosome solution, mix thoroughly and incubate for 3-5 minutes at room temperature.
- 3) Transfer the mixture of Lysis Buffer and exosomes to DNA Clear Column.
- 4) Centrifuge at 12, 000 g for 2 min at 4 °C, discard DNA clear column.
- 5) Add equal volume of isopropyl alcohol (4 ml) to the filtrate, and mix it upside down.
- 6) Add 50 µL magnetic beads (vortex the magnetic beads before use), mix thoroughly, and incubate for 30 minutes at 4 °C by using a rotary mixer.
- 7) Pellet magnetic beads by centrifugation at 3000 g for 5 min, or by using the magnetic rack. Carefully remove and discard the supernatant.
- 8) Add 1 ml Wash Buffer C, vortex at medium speed for 30 sec to completely disperse the magnetic beads.
- 9) Pellet the magnetic beads by centrifugation or using the magnetic rack. Carefully remove and discard the supernatant.
- 10) Add 1 ml Wash Buffer D, vortex at medium speed for 30 sec to completely disperse the magnetic beads.
- 11) Pellet the magnetic beads by centrifugation or using the magnetic rack. Carefully remove and discard the supernatant.
- 12) Centrifuge at 3000 g for 1 min, and remove the residual liquid with a pipette.
- 13) Add 100 µL Elution Buffer R (or RNase-free ddH₂O), and vortex for 30 sec.
- 14) Centrifuge at 7000 g for 2 min, and then put the centrifuge tube on a magnetic rack, and then transfer the supernatants to new tubes, which is the microRNA solutions.
- 15) The isolated microRNA may be used to measure concentration immediately or stored at -80 °C.

6. Identification of Isolated microRNA

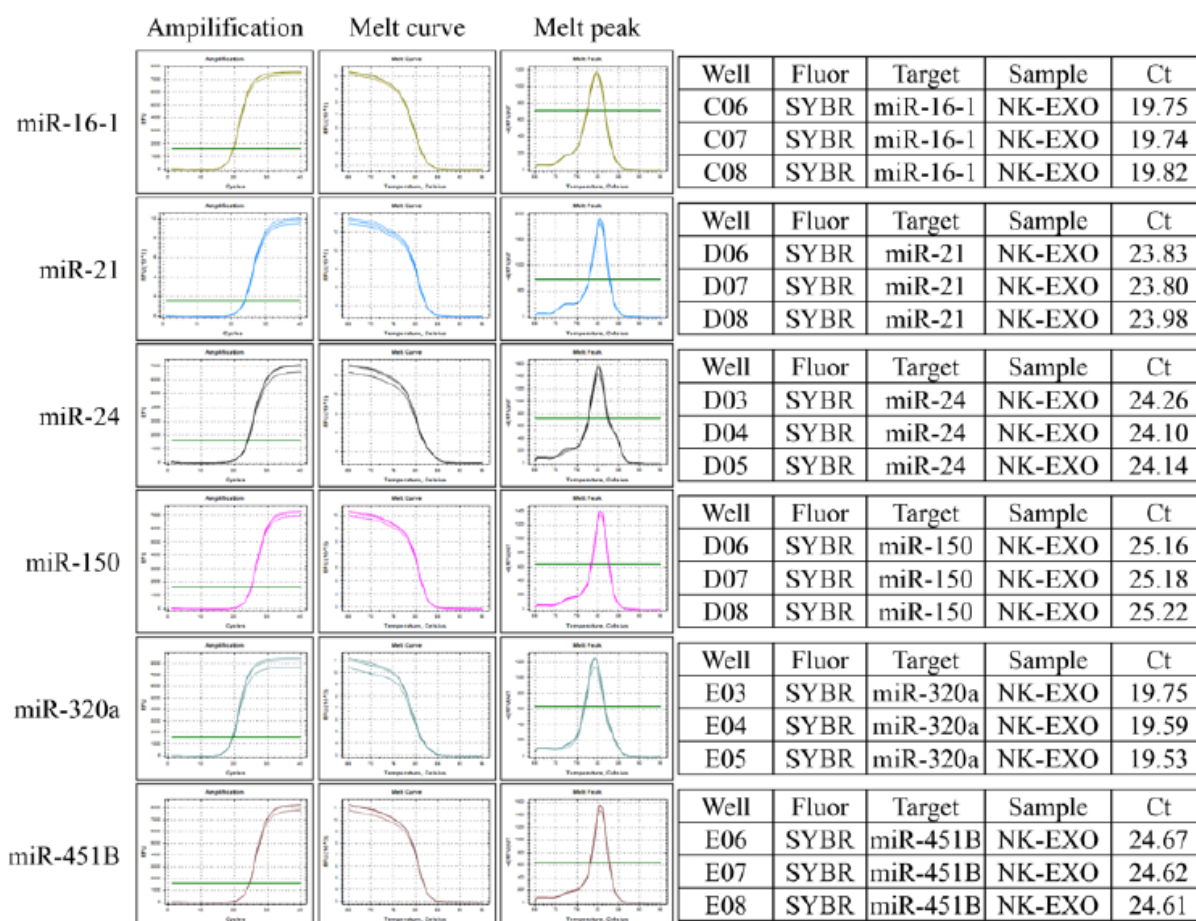


Figure 1. Identification of microRNA isolated from exosomes (purified from NK cells culture supernatant) by using QPCR.

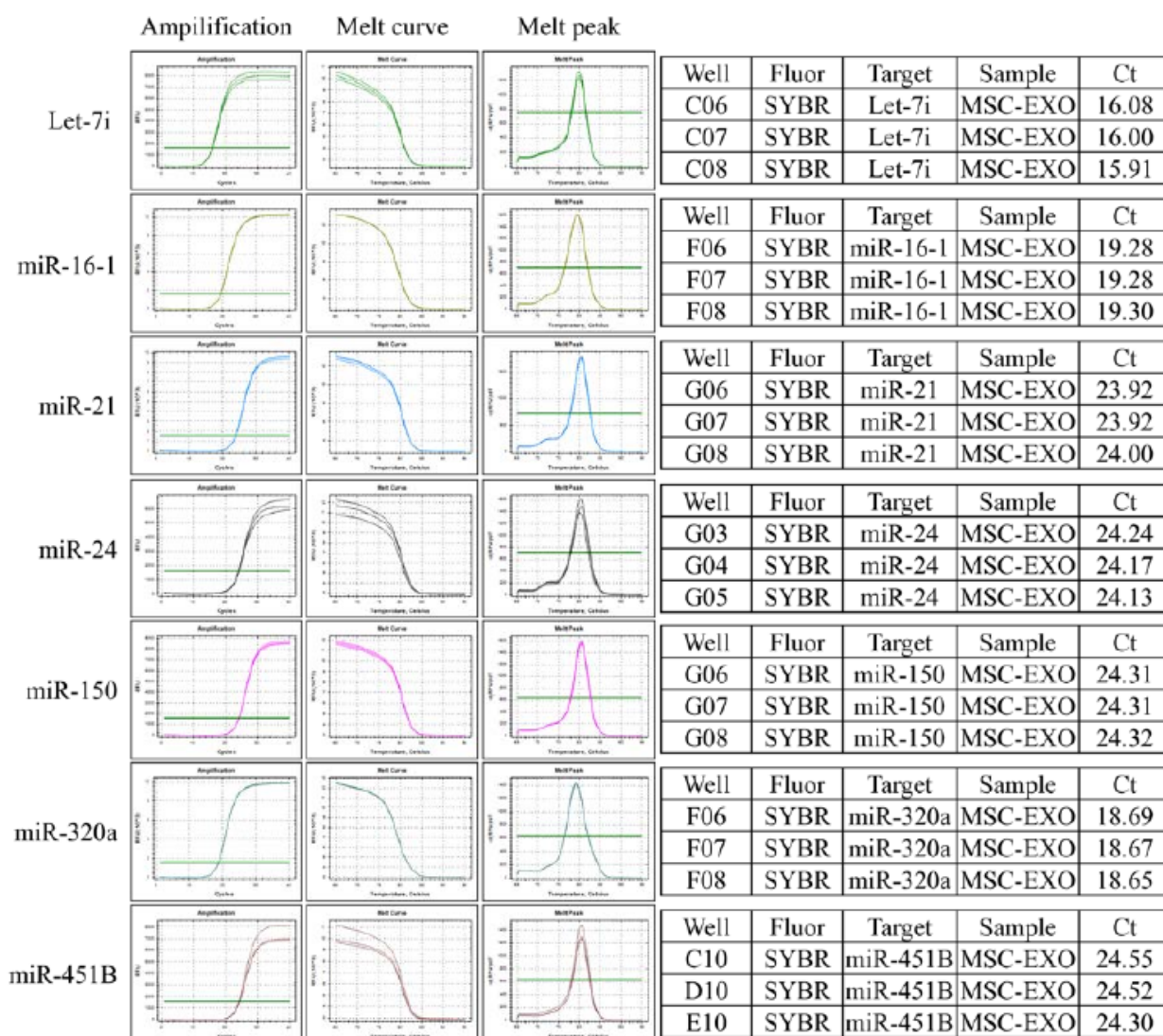


Figure 2. Identification of microRNA isolated from exosomes (purified from mesenchymal stem cells culture supernatant) by using QPCR.

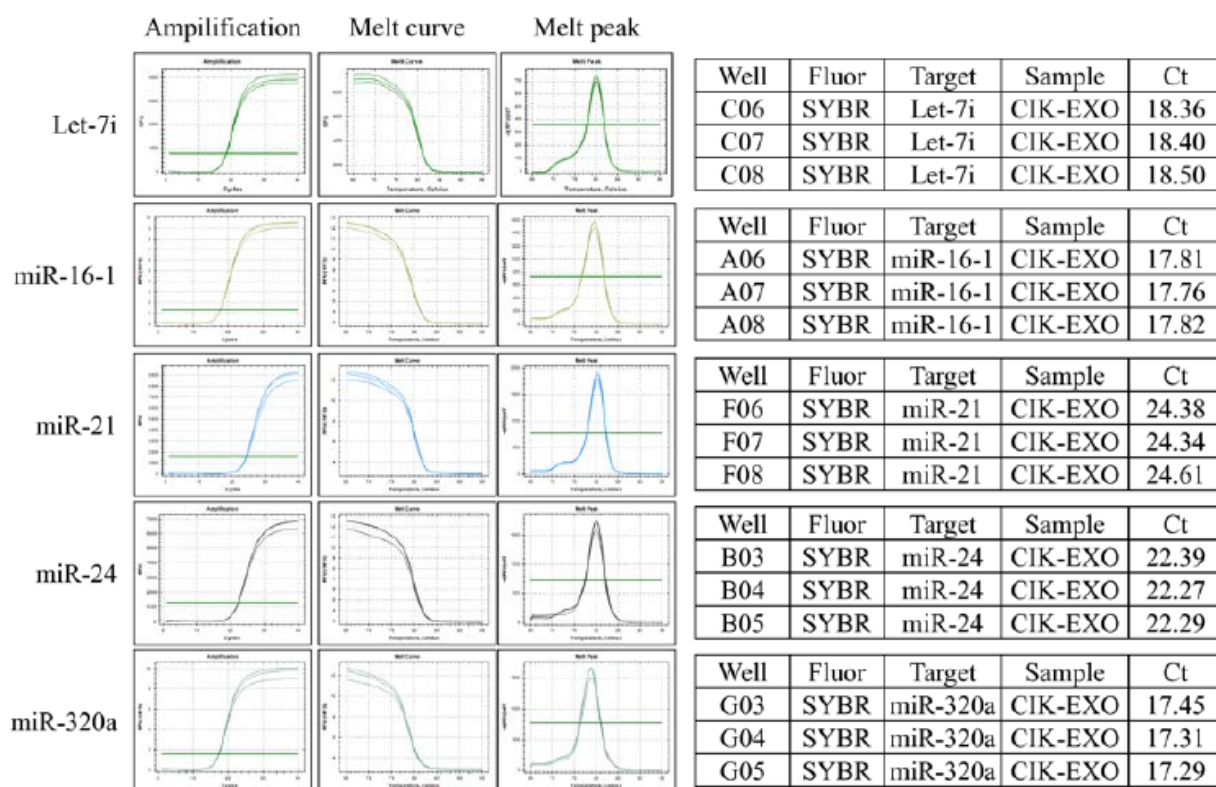


Figure 3. Identification of microRNA isolated from exosomes (purified from cytokine-induced killer cells culture supernatant) by using QPCR.

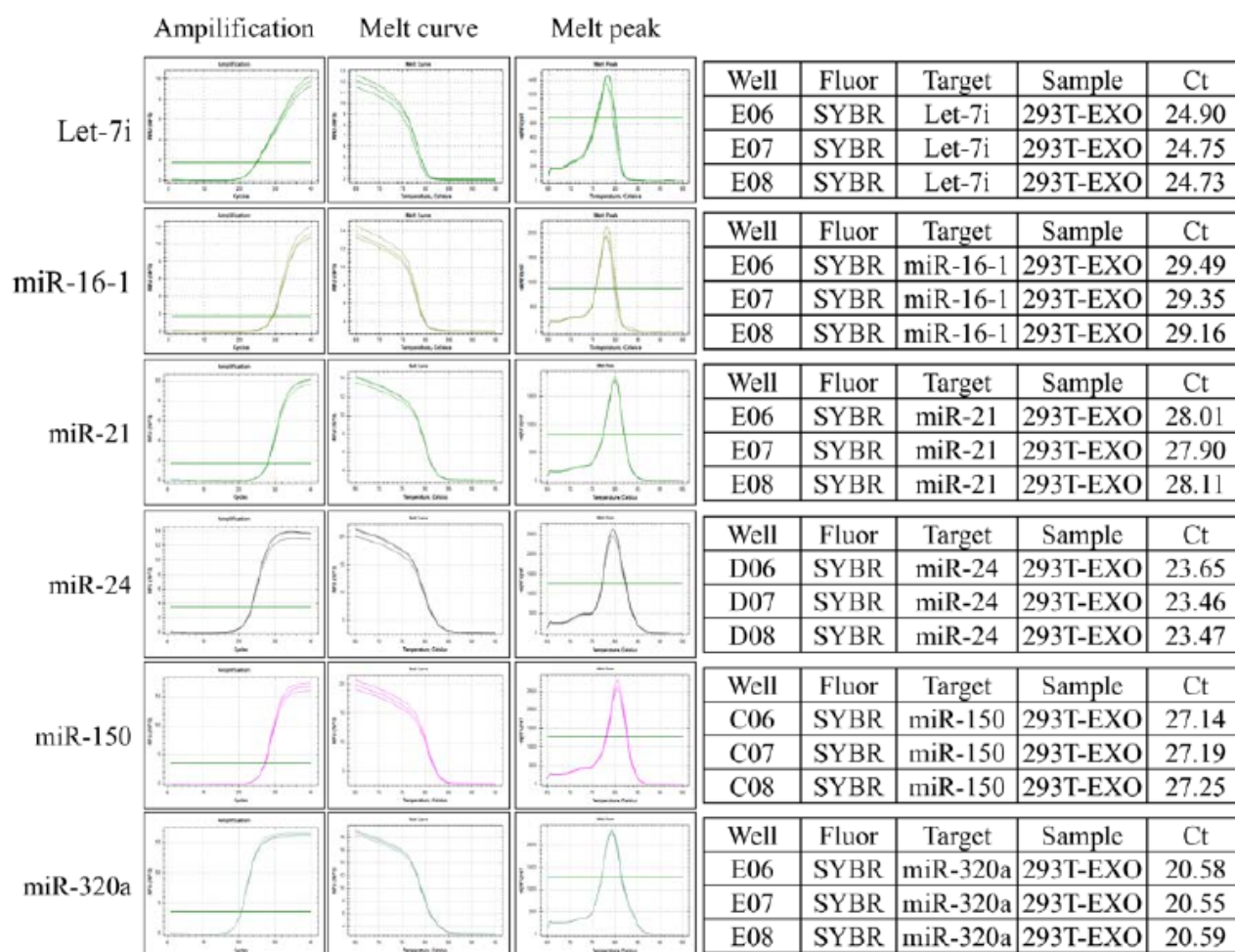


Figure 4. Identification of microRNA isolated from exosomes (purified from 293T cells culture supernatant) by using QPCR.

7. Frequently Asked Questions and Analysis

Questions	Reasons	Solutions
The isolated microRNA has been degraded	The exosomes have been frozen and thawed repeatedly.	Use fresh exosomes to isolate microRNA.
	The exosomes have not been stored below -80 °C.	Store the exosomes at -80 °C or in the liquid nitrogen
	The reagents and materials used in the experiment are not RNase-free.	Use RNase-free reagents, tubs and tips.
	The microRNA isolation process does not carry out on ice.	Isolate microRNA from exosomes on ice.
	The elution buffer for microRNA is not RNase-free.	Use RNase-free elution buffer or ddH ₂ O to elute microRNA from magnetic beads.
	The storage of microRNA is improper.	The isolated microRNA should be stored at -80 °C, but not -20 °C.
The concentration of isolated microRNA is low.	Less exosomes were used in microRNA isolation.	Increase exosomes for microRNA isolation.
	The concentration of exosomes is low.	Use exosomes with high concentration to isolate microRNA.
	The intensity of elution is not enough to elute microRNA.	Increase vortex intensity and prolong vortex time.
	The magnetic beads become invalid and cannot absorb microRNA.	Use new magnetic beads.
	More elution buffer was used to elute microRNA.	Reduce elution buffer used in the experiment.