

EXOBX® Exosome Purification Kit (PLUS)

Cat. no. R6303E

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Catalog Number	Reagent In Kit	Size	Storage
R6303E	Buffer EXA	40 mL	-20°C
	Buffer EXB	10 ml	4°C
	Buffer EXE	10 ml	4°C
	Magnetic Beads	3.5 ml	4°C
	Needle filter A	10	RT
	Needle filter B	10	RT
	Manual	1	

1. Transportation and Storage

This product is transported at low temperature, valid for 2 years.

2. Important Product Information

- 1) The magnetic beads should be stored at 4 °C and are forbidden to freeze, or they become invalid.
- 2) PBS and ddH₂O used in the experiment must be filtered by a filter with a pore size of 0.2 µm.
- 3) Magnetic beads will sink to the bottom of the tube after standing for a long time, so vortex the magnetic beads before sucking them.
- 4) This kit is not suitable for extracting exosomes from highly concentrated cell culture medium.
- 5) It is recommended to pre-rinse the syringe filter with PBS before filtering exosomes to minimize the loss of exosome solution.
- 6) The purified exosomes can be stored at -80 °C, and should be avoid repeat freezing and thawing.
- 7) The exosome purification system provided by this kit is 20 ml/ sample. If there are few samples, ddH₂O can be used to fill the volume.
- 8) Please wear lab clothes, gloves and safety glasses during the experiment.

3. Product Introduction

The product was designed to isolated exosomes from cell culture mediums by using magnetic beads, which adsorb exosomes in the purification system and release exosomes in the elution buffer. Moreover, the exosomes eluted by Buffer EXE could be used in cell culture and animal injection.

4. Product Features

- High-efficiency purification with excellent recovery of exogenous nucleic acids.

- The obtained exosomes exhibit high purity and low levels of contaminating proteins.
- Easy to use and enables rapid exosome purification within 2 hours.
- The purification procedure is gentle, preserving the integrity of the exosomes.
- The obtained exosomes are free of other chemical contaminants and can be directly used for cell culture experiments and in vivo injection studies.

5. Experimental Procedure

- 1) This kit is suitable for isolating exosomes from cell culture mediums.
- 2) Sample preparation. Centrifugate cell culture supernatant at 3000 g for 10 min at 4 °C, collect the supernatant to a new tube, and then 10000 g for 10 min at 4 °C, transfer the supernatant to a new tube and discard the precipitate.
- 3) Moisten and wash needle filters A by using 5 ml PBS, and then filter the suspension collected in the previous step by using needle filter A to a new tube.
- 4) Preparation of magnetic beads. Vortex the magnetic beads for 30 sec, transfer 0.35 ml magnetic beads to a 50 ml centrifuge tube, centrifuge at 3000 g for 2 min at 4 °C, and discard the supernatant.
- 5) Magnetic bead washing. Add 10 ml PBS precooled at 4 °C, vortex for 30 sec, centrifuge at 3000g for 5 min at 4 °C, and discard the supernatant.
- 6) Exosomes purification system. The exosome purification system provided by this product is 20 ml, and the components are as follows.

Table 1. Exosome purification system

Reagents	Size(ml)	Proportion
Buffer EXA	4	20 %
Buffer EXB	1	5 %
Magnetic Bead	0.35	1.75 %
cell culture mediums	14.65	73.25 %
Total	20	100 %

- 7) Prepare the exosome purification system according to table 1, if the sample volume is less than 14.65 ml, use ddH₂O to fill the full system.
- 8) Mix the exosome purification system in the tubes by using a rotary mixer at 4 °C for 40 min.
- 9) Pellet magnetic beads by centrifuging at 3000 g for 5 min at 4 °C, or by using the magnetic rack.
Carefully remove and discard the supernatant.

- 10) Centrifuge at 3000 g for 1 min at 4 °C, and remove the residual liquid with a pipette.
- 11) Add 0.5-1.0 ml Buffer EXE (when the eluent volume is large, the exosome concentration is low, otherwise, it is high), and vortex for 30 sec.
- 12) Centrifugate at 7000 g for 2 min at 4 °C, and then put the centrifuge tube on a magnetic rack, and then transfer the supernatants to new tubes, which is the exosome solutions.
- 13) Moisten and wash needle filters B by using 5 ml PBS, and then filter exosomes solutions by using needle filters B subsequently.
- 14) The purified exosome solution can be immediately used for detection or stored at -80 °C.

6. Identification of Isolated exosomes

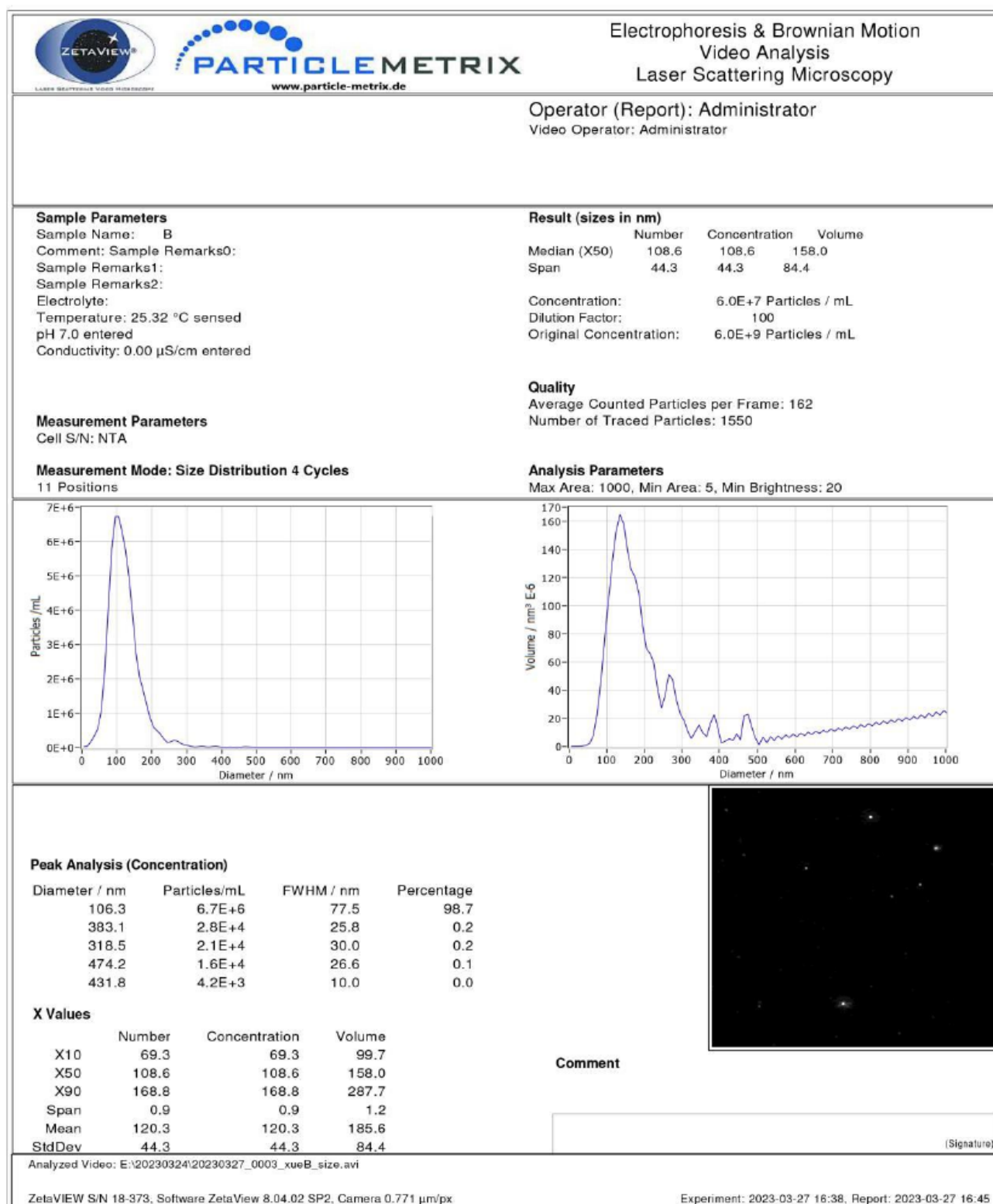


Figure 1. Identification of exosomes (purified from the culture medium of mesenchymal stem cells) by using NTA analysis.

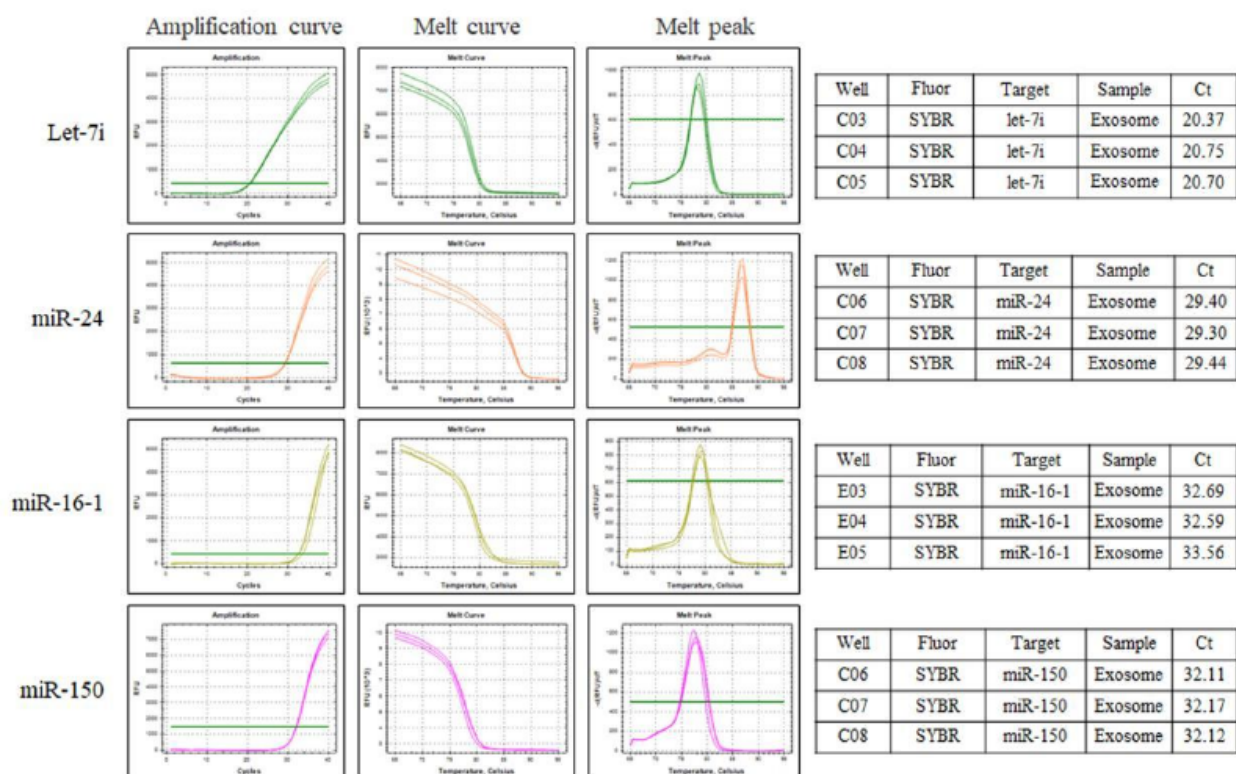


Figure 2. Determination of miRNA in exosomes isolated from CIK cell culture medium (QPCR method)

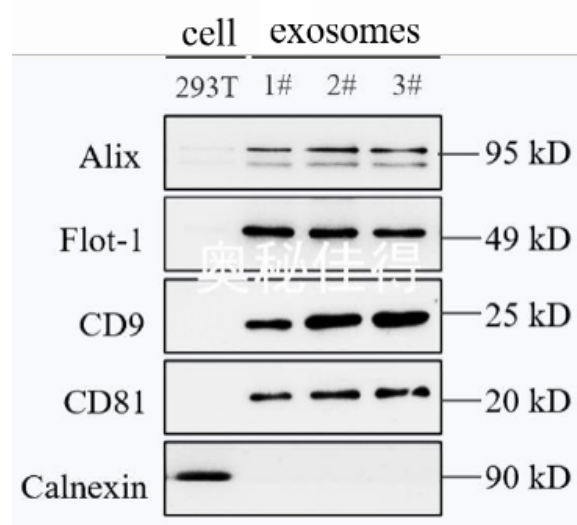


Figure 3. Identification of exosomes marker protein in exosomes purified from cells culture medium.

7. Frequently Asked Questions and Analysis

Questions	Reasons	Solutions
The concentration of isolated exosomes is low.	Samples contain less exosome.	Use samples contain more exosomes, or concentrate the samples.
	Less sample was used to purify exosomes.	Increase the samples used in exosome purification.
	The samples have been frozen and thawed repeatedly.	Use fresh sample to purify exosomes.
	Samples were not stored at low temperature.	Samples should be stored at -80 °C for long time.
	Samples were filtered repeatedly.	Don't filter samples.
	Reagent becomes invalid.	Use the reagents within the shelf life.
	Magnetic beads have been frozen and become invalid.	Use new magnetic beads.
	Magnetic beads were reduced in the purification system.	Add the exact volume magnetic beads according to the manual.
	The proportion of reagents in the purification system changed.	Add the reagents to the purification system according to the requirement strictly.
	Not following the instructions provided in the manual.	Incubation mode and elution intensity should be carried out in strict accordance with the instructions.
	The incubation time between samples and magnetic beads is short.	Prolonged incubation time.
	Elution intensity is low.	Increase vortex intensity and prolong vortex time.
	Eluent volume is too large.	Reduce the volume of eluent.
The concentration of exosome protein is low.	The concentration of isolated exosomes is low.	Increase exosome concentration.
	The lysis buffer is weak for lysing exosomes.	Select the lysis buffer with strong protein denaturant.
	The isolated exosomes contain less foreign protein.	Increase exosomes for lysing.

The foreign proteins in the isolated exosome increase	The incubation time between the samples and magnetic beads is too long, which leads to nonspecific adsorption of foreign proteins.	Reduce the incubation time between samples and magnetic beads.
	The samples have been concentrated, so the foreign protein increases in the sample.	Reduce the amount of sample used for exosome purification, or use unconcentrated samples.
The miRNA isolated from purified exosomes is low.	The exosomes concentration is low.	Increase exosome concentration.
	Less exosomes were used for miRNA isolation	Increase the amount of isolated exosomes used in miRNA isolation.
	The RNA purification kit is not suitable for isolating miRNA from exosomes.	Select the suitable kit for exosome miRNA isolation.