ACSBIOMARKER

Technote

Exosome Extraction

Exosomes

Exosomes are nanosize microvesicles shed by cells into the blood and other body fluids as a way of cell-to-cell communication. Exosomes are particles of ~50-100nm in diameter with a bi-lipid membrane containing significant amounts of microRNA, proteins and lipids unique to the cell of origin under normal or pathological conditions. Exosomes are rich in disease-specific biomarkers that can be isolated from the plasma and serum and do not suffer from the noise of abundant plasma proteins in a diagnostic test.

Electron Microscopy

Successful exosome enrichment from plasma and serum samples.

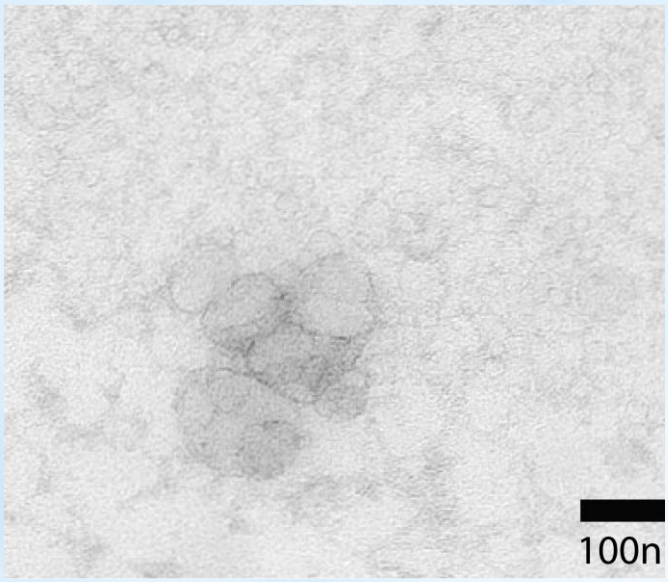


Figure 1. A scanning electron microscopy (SEM) image of exosomes isolated from serum.

Sample Types

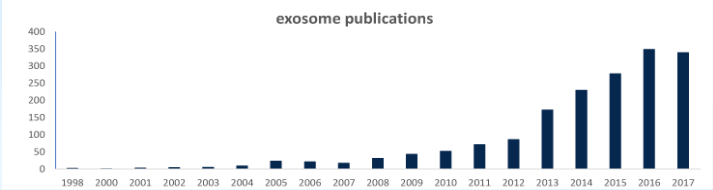
Our exosome isolation protocol is optimized for serum and anticoagulant-treated blood samples only. Untreated samples contain high levels of clotting factors that will interfere with the isolation process resulting in impurities and a lower yield. Anticoagulant treated tubes such as EDTA-, Heparin-, or Citrate treated are commercially available and must be used during blood collection following the manufacturer's protocol.

What do we need?

- Plasma or serum samples (-80°C)
- Volume 150µl
- Information on downstream application:
  - RNA profiling
  - Protein analysis

Growing interest in exosomes

In recent years the number of exosome biomarker studies is steadily increasing indicating the growing interest and value of biomarker studies using samples enriched for exosomes



Technote – Exosome Extraction

Protein Analysis

Samples enriched for exosomes contain concentrated amounts of exosome specific proteins. These samples can be used for downstream applications such as Western Blotting or ELISA protein measurements

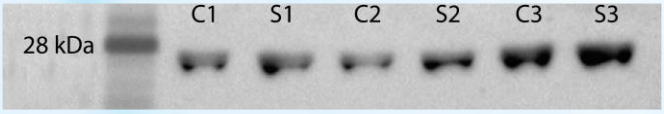


Figure 2. Western blot analysis performed on exsome enriched plasma and serum samples. Each band corresponds to CD9-specific exosomal protein marker at ~25 kDa. *From left to right:* Protein ladder in KDa, C1 (citrate plasma patient #1), S1 (serum patient #1), C2, S2, C3, S3.

miRNA Analysis

Samples enriched for exosomes contain concentrated amounts of exosome specific miRNAs. Exosome enriched samples can be used for qPCR analysis of miRNAs

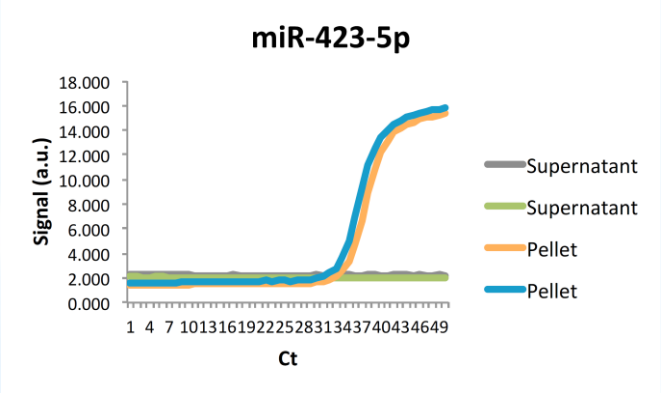


Figure 6 . A representative example of miRNA cycle times qPCR of a miRNA following exosome isolation is shown. miRNAs were isolated from supernatant and exosome pellets using TRIzol®. After cDNA synthesis miRNAs were amplified and detected using miRNA specific forward primers and universal reverse primers using SYBR Green reagents on a LightCycler® 480.

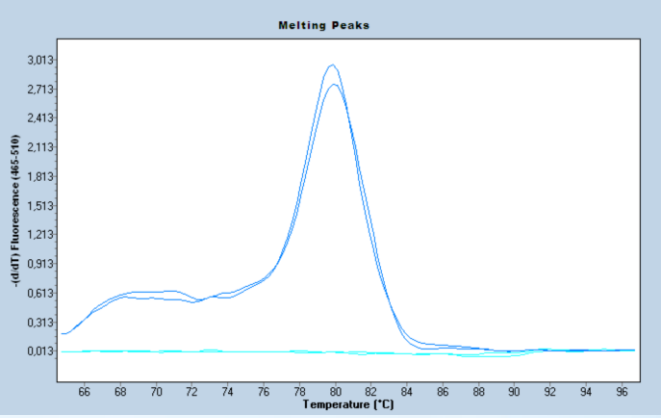


Figure 7. A representative example of the qPCR melt curve for a miRNA following exosome isolation is shown.