



Amplify Exosome RNAs from Serum

Travis Antes, PhD and Nandita Sarkar, PhD

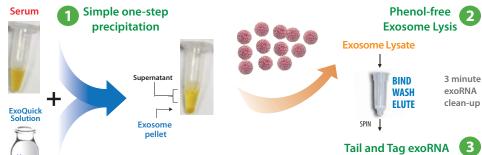
Exosomes - MicroRNAs - Cancer Biomarkers

RNAs present in patient body fluids are a rich and untapped source of disease-related biomarkers. The RNAs are stable in serum because they are encapsulated in circulating exosomes. Exosomes are 40-100 nm membrane vesicles secreted by most cell types in vivo and in vitro. Exosomes are found in blood, urine, amniotic fluid, malignant ascite fluids and contain distinct subsets of microRNAs depending upon the tumor or tissue from which they are secreted. In this study, SBI utilizes a proprietary polymer mixture (ExoQuick) to precipitate exosomes from human serum samples. Exosome precipitation from serum samples was verified by Western blot using antibodies against two commonly found exosome biomarker proteins, CD63 and CD9 and visualized using EM and NanoSight technologies. The exosome pellet was then processed for RNA amplification using SBI's SeraMir Kit. The SeraMir kit includes everything needed to accurately and sensitively measure RNAs from serum samples. Exosomes are efficiently isolated using SBI's ExoQuick solution, and the exoRNAs are purified using a phenol-free lysis buffer and rapid spin columns. The SeraMir kit enables the 3' tailing and simultaneous tagging of both 5' and 3' ends during cDNA synthesis - ready for qPCR. Primers for PCR amplification are included for highly sensitive applications. We present a new approach using ExoQuick and SeraMir technologies to facilitate high-throughput exosome RNA analysis which will enable major strides forward in circulating microRNA biomarker discoveries for cancer diagnostic and prognostic tools.

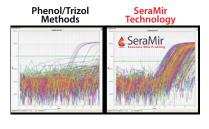
Highlights

- Exosomes are released from tumors in high abundance
- Exosome cargo reflects the origin and physiological state of the source cells
- MicroRNAs are found in high abundance in circulating exosomes
- ExoQuick easily precipitates exosomes from patient bio-fluids
- Higher purity exosome recovery with a simple protocol
- Tail and Tag both ends of exoRNAs for efficient amplification

SeraMir™ exoRNA Amplification - How does it work?

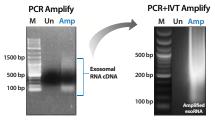


- Precipitate exosomes patient serum with ExoQuick
- Purify exoRNAs with SeraMir columns
- Tail and tag all exoRNAs for qPCR, cDNA amplification and T7 IVT
- Amplify exoRNAs for Microarrays and NextGen Sequencing
- Perform qPCR profiling on cDNA or use T7 IVT of exoRNAs for Arrays and NGS



Serum RNA prepared by conventional Trizol versus the SeraMir kit. Profiling of 380 Human microRNAs.

Formal RNA 5'Switch Adaptor 5' Adaptor Formal RNA Polyadenylate AAAAAAAAAA RT reaction 3' adaptor primer 5' T7 - 3' 3' 5' 3' 5'



SeraMir's ExoQuick™ Easily Precipitates Exosomes

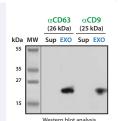


 No time-consuming ultracentrifugation

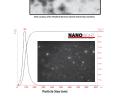
Less expensive than costly
 DynaBeads

 More effective than any other method

• Use as little as 250µl of serum



ExoQuick Exosome Analyses



www.systembio.com/seramir