Purified Exosomes from Human Biofluids (Serum, Saliva, Urine, and CSF)

Cat# EXOP-500A-1, EXOP510A-1, EXOP-520A-1, EXOP-530A-1

User Manual

Store at -20^{0}C

Version 1
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Contents

Product Description

SBI's purified exosomes from pooled human biofluid samples come from healthy donors and include exosomes isolated from serum, urine, CSF, and saliva, with more biofluids on the way. Each lot of exosomes is carefully characterized for particle size and concentration by NanoSight analysis, and expression of specific exosome protein markers validated by western blot.

Purified exosomes are useful for a variety of applications, including the following:

- Protein biomarker analysis
- qPCR for RNA biomarkers
- High-throughput biomarker discovery (e.g. Mass Spec)
- Electron microscopy
- Standardized controls for disease studies

Each vial of purified exosomes contains >25ug of exosomal protein (as measured by Qubit assay) resuspended in 1X PBS solution and is ready to use. Each vial comes from lots that have been QC'd by NanoSight analysis for particle concentration and size as well as western blotting for specific markers, ensuring quality reagents for your mission-critical exosome experiments.

List of Components

Item	Amount	Storage Temperature
Purified Exosomes from Human Biofluids	>25ug	-20^{0}C

Storage

Purified exosomes are shipped in dry ice and should be **stored** at -20°C. Properly stored kits are stable for 1 year from the date received.

Suggested Protocol (Western Blot analysis) for Purified Exosomes

SDS-PAGE:

1. Add right amount of Lane Marker Reducing Buffer to 5-10 ug of your samples to get 1X working concentration of buffer in a total amount of 15 or 50 ul (10-well or 15-well gel respectively)

Optional step: Lyse the samples with proper lysis buffer before adding reducing reagent

2. Incubate the samples at 95˚C for 5 min
3. Put the tubes on ice to cool down the samples, then spin down for 10 sec
4. Prepare the gel/running buffer and load the samples
5. Run the gel at 100 V for at least 45-60 min.

Western Blot:

6. Remove the gel cassette and break it to take the gel out
7. Wet the filter papers, sponges, and Nitrocellulose membrane with cold Transfer Buffer and start making the sandwich.
8. Put an ice pack in the tank and run it at 100 V for at least 45 min.
9. Block the membrane with SuperBlock T20 (PBS) Blocking Buffer (or 5% milk powder in TBST buffer) on shaker for 1-2 hours.
10. Discard the blocking buffer, pour primary antibody solution (1:1,000) on membrane and incubate it at 4˚C overnight on a shaker.
11. Wash the membrane with TBST four times each 10 min on a shaker.
12. Add secondary antibody solution (1:15,000) on membrane and incubate it at room temperature for one hour on a shaker.
13. Wash the membrane with TBS-T four times each 10 min on a shaker.
14. Image the membrane with ChemiDoc instrument using SuperSignal West Femto substrate

Next Steps and Related Products

Application	Related Products	Website links
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck™ Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays
Quantification of Exosomes		
Quantification of exosomes	FluoroCet assay	https://www.systembio.com/quantitate-exosomes/fluorocet

Quantification of exosomes	ExoELISA-ULTRA assay	https://www.systembio.com/quantitate-exosomes/exoelisa-ultra
Quantification of exosomes by fluorescent NanoSight	ExoGlow-NTA kit	https://www.systembio.com/exosome-research/exoglow-nta/overview
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir™ kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

Figure 1. Western blot results for exosome-specific protein markers from purified human exosomes

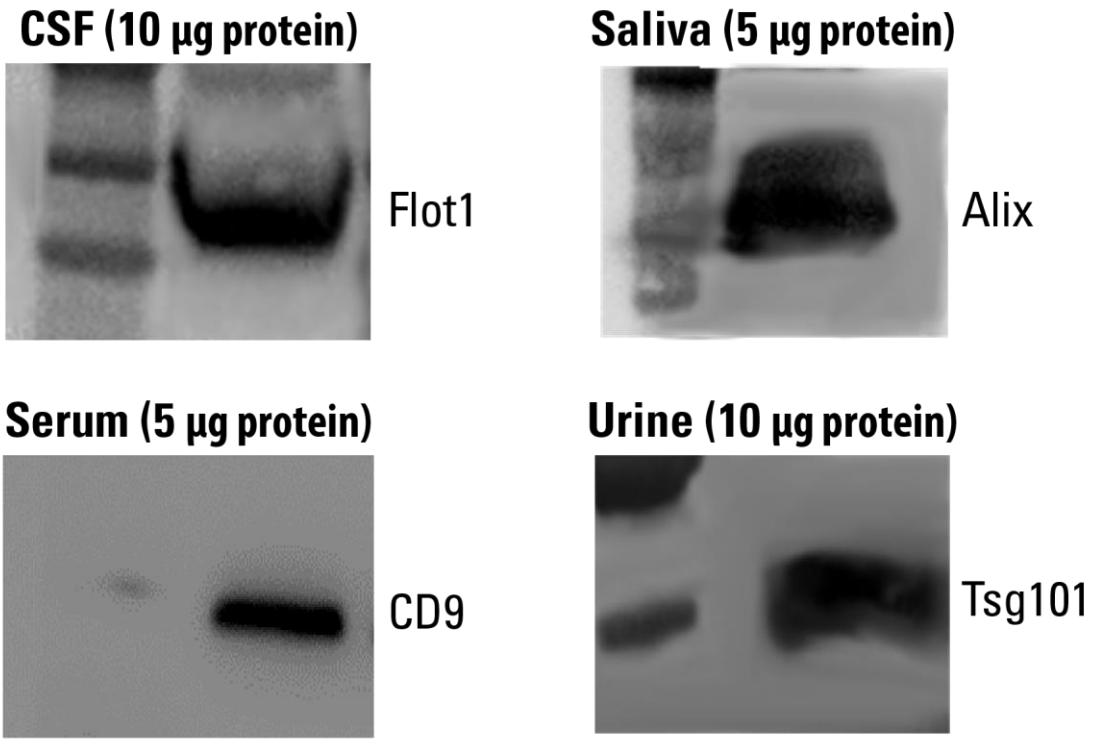
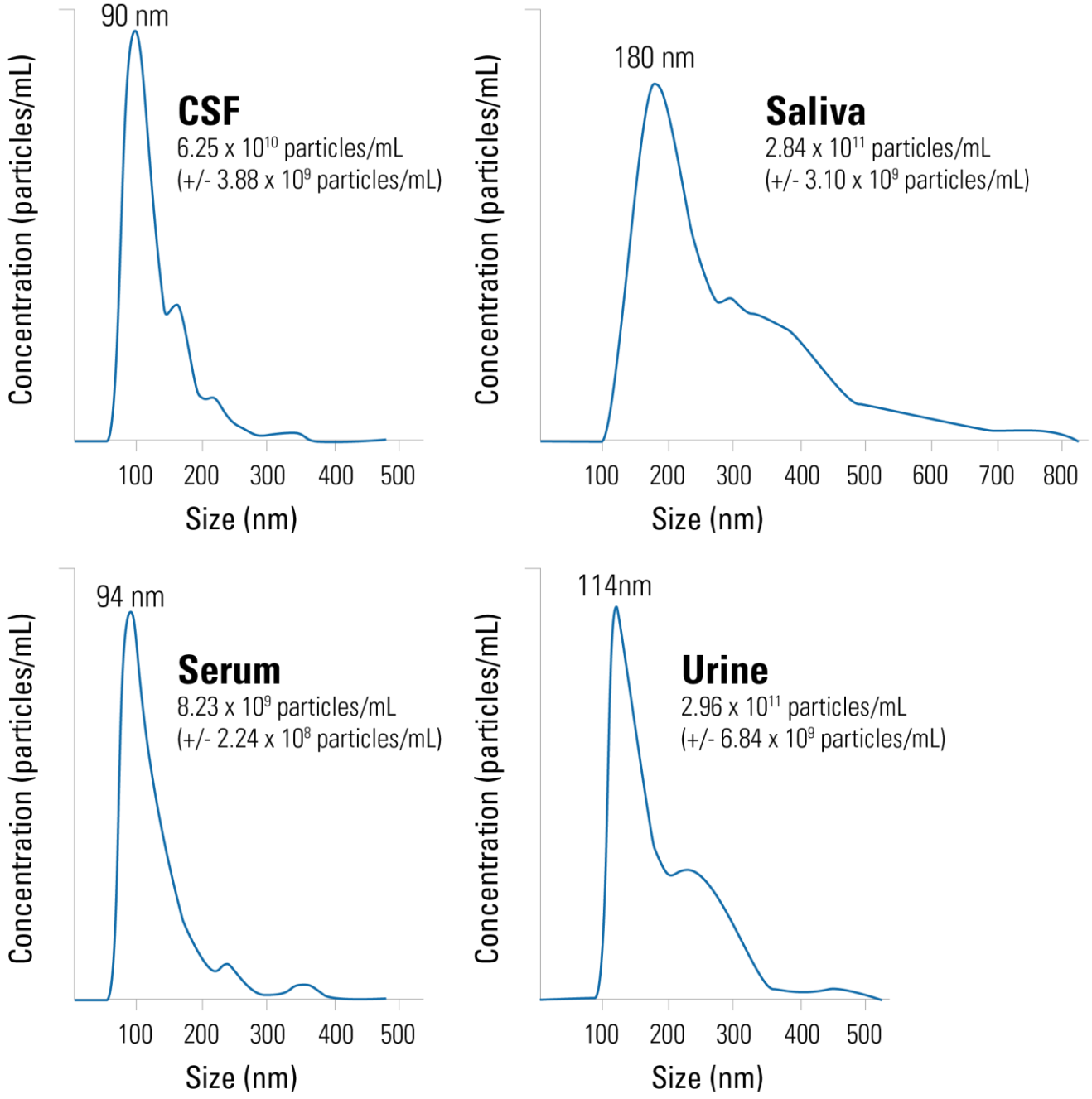


Figure 2. NanoSight analysis of purified human exosomes showing approximate particle size distribution (x-axis) and particle concentration (particles/ml) (y-axis). Average particle concentration and CV, and the mode of particle size are shown for each exosome sample



Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

For additional information or technical assistance, please call or email us at:

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