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°C

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the License and Warranty Statement contained in this user manual.
Contents

Product Description ........................................................................................................................................ 2
List of Components ..................................................................................................................................... 2
Storage ......................................................................................................................................................... 2
Suggested Protocol (Western Blot analysis) for Purified Exosomes ......................................................... 3
Next Steps and Related Products ............................................................................................................... 3
Example Data and Applications .................................................................................................................. 4
Technical Support ........................................................................................................................................ 6
Licensing and Warranty Statement ............................................................................................................... 6
**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**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Exosomes from Human Biofluids</td>
<td>&gt;25ug</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Application</th>
<th>Related Products</th>
<th>Website links</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Characterization of Exosomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western blotting</td>
<td>Exosome antibodies</td>
<td><a href="https://www.systembio.com/microrna-research/exosome-antibody/exoab">https://www.systembio.com/microrna-research/exosome-antibody/exoab</a></td>
</tr>
<tr>
<td>Antibody Arrays</td>
<td>ExoCheck™ Assays</td>
<td><a href="https://www.systembio.com/microrna-research/exosome-antibody-arrays">https://www.systembio.com/microrna-research/exosome-antibody-arrays</a></td>
</tr>
<tr>
<td>Quantification of Exosomes</td>
<td>FluoroCet assay</td>
<td><a href="https://www.systembio.com/quantitate-exosomes/fluorocet">https://www.systembio.com/quantitate-exosomes/fluorocet</a></td>
</tr>
</tbody>
</table>

3
<table>
<thead>
<tr>
<th>Quantification of exosomes</th>
<th>ExoELISA-ULTRA assay</th>
<th><a href="https://www.systembio.com/quantitate-exosomes/exoelisa-ultra">https://www.systembio.com/quantitate-exosomes/exoelisa-ultra</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantification of exosomes by fluorescent NanoSight</td>
<td>ExoGlow-NTA kit</td>
<td><a href="https://www.systembio.com/exosome-research/exoglow-nta/overview">https://www.systembio.com/exosome-research/exoglow-nta/overview</a></td>
</tr>
</tbody>
</table>

**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

- **CSF (10 µg protein)**: Flot1
- **Saliva (5 µg protein)**: Alix
- **Serum (5 µg protein)**: CD9
- **Urine (10 µg protein)**: Tsg101
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:

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax (650) 968-2277
E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

Licensing and Warranty Statement

Limited Use License
Use of Purified Exosomes from Human Biofluids (i.e., the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

• The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

• The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

• This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI’s liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI’s liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.
SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.
This page is intentionally blank