

EV-Guard[™] EV Storage Buffer

Cat # EXSBA-1, Cat # EXSBA-10

User Manual

Please see individual components for storage conditions

Version 1 5/23/2023

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

Product Description	1
List of Components	
Storage	
General Information	
Protocol: Using the EV-Guard TM EV storage buffer – 1X	
Protocol: Using the EV-Guard™ EV storage buffer – 10X	
Example Data and Applications	
Related Products	
Technical Support	
Licensing and Warranty Statement	6

Product Description

EV-Guard™ EV Storage Buffer (EV-Guard™) is an innovative solution designed to safeguard extracellular vesicles (EVs) and maintain their biological activities during storage. With its advanced formulation, EV-Guard™ effectively preserves the integrity and stability of EV under various storage conditions, including multiple freeze-thaw cycles. Whether stored at 4°C, -20°C or -80°C, EV-Guard™ shields EVs from degradation and ensures their optimal performance.

List of Components

ltem	Catalog #	Volume
EV-Guard™ EV storage buffer – 1X	EXSBA-1	40 ml
EV-Guard [™] EV storage buffer − 10X concentrated	EXSBA-10	4 ml

Storage

The EV-Guard[™] EV Storage Buffer are shipped on blue ice or at ambient temperature and should be stored at +4°C upon receipt. Properly stored kits are stable for 1 year from the date received.

General Information

The buffer has been sterilized by $0.22~\mu m$ membrane filter. The buffer is designed in both 1X and 10X concentrated formats to suit the needs for customers who choose to use different exosome isolation methods which result in concentrated EV pellets (e.g. by ultracentrifugation, ExoQuick or other precipitation based methods) or exosome suspensions (e.g. by SmartSEC or other size exclusion based methods).

Protocol: Using the EV-Guard[™] EV storage buffer – 1X

- 1. Resuspend purified EV pellet in 100 ul 500 ul of 1X EV-Guard™ EV storage buffer.
- 2. **Optional:** Perform a protein quantitation assay (e.g. BCA or Qubit assay) to determine the amount of protein in your sample.
- 3. Aliquot EV samples into appropriate volumes for storage.

Protocol: Using the EV-Guard[™] EV storage buffer – 10X

- 1. Add 10X concentrated EV-Guard[™] EV storage buffer to purified EV suspension to a final concentration of 1X. Gently pipet to mix.
- 2. **Optional:** Perform a protein quantitation assay (e.g. BCA or Qubit assay) to determine the amount of protein in your sample.
- 3. Aliquot EV samples into appropriate volumes for storage

Example Data and Applications

EV-Guard™ EV Storage Buffer prevents loss of EV particles after multiple freeze-thaw cycles.

EVs were isolated from HEK293T cell culture media using SBI's ExoQuick-TC. The samples were stored at -80°C in either PBS or EV-Guard™ before undergoing single and multiple freeze-thaw cycles. The size distribution and concentrations of EV particles were determined by nanoparticle tracking analysis (NTA) (Figure 1A). Compared to the samples stored in PBS, which experienced approximately a 30% loss after five freeze-thaw cycles, the EVs stored in EV-Guard™ exhibited 98% conservation (Figure 1B).

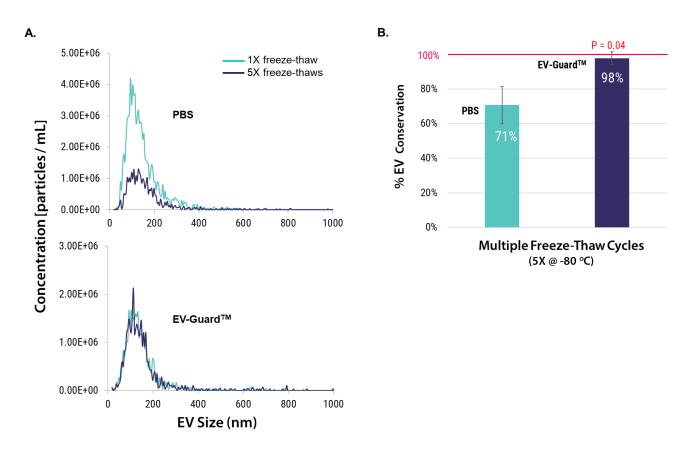


Figure 1. The size distribution (A) and the percentage of EV conservation (B) were assessed by NTA following multiple freeze-thaw cycles in both EV-GuardTM buffer and PBS. The data were derived from five independent experiments (n=5), each compared to a single freeze-thaw cycle.

EV-Guard[™] EV Storage Buffer minimizes loss and aggregation of EV particles during long-term storage

EVs from normal human serum were isolated using SBI's ExoQuick and stored at 4°C for intervals of 0 days, 15 days, and 30 days. Subsequent analyses were conducted using fluorescent nanoparticle tracking analysis (fNTA). It was observed that EV samples preserved in EV-Guard™ buffer exhibited a higher percentage of EV conservation than samples preserved in PBS after both 15 and 30 days (Figure 2A). Furthermore, the overall sizes of the EVs were consistently maintained during storage in EV-Guard™, but showed variation when stored in PBS (Figure 2B).

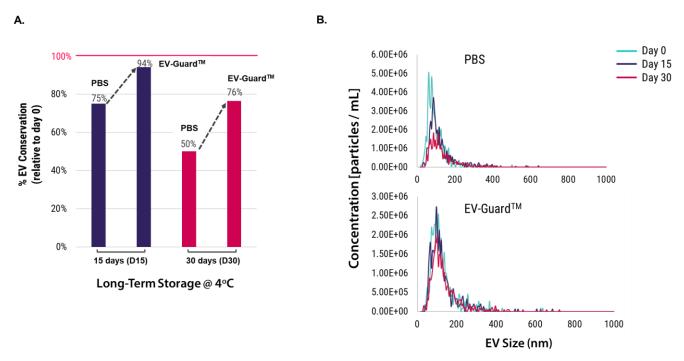


Figure 2. EVs from normal human serum were isolated using SBI's ExoQuick and stored at 4°C for periods of 0, 15, and 30 days. The percentage of EV conservation (A) and EV size distribution (B) were subsequently analyzed using fluorescent nanoparticle tracking analysis (fNTA).

EV-Guard™ EV Storage Buffer preserves EV functionality and integrity after long-term storage

GFP-loaded EVs were isolated from the Xpack CMV-XP-GFP-EF1a-Puro Stable HEK293 Producer Cell Line (Cat. XPAK530CL-1) using SBI's ExoQuick-TC. These EVs were stored in either PBS or EV-GuardTM at -20°C for 1 month before being introduced to HEK293 cells for transfection. After 1 month at -20°C, the GFP-loaded EVs in EV-GuardTM demonstrated higher transfection efficiency (Figure 3, C4) than those stored in PBS (Figure 3, C2). Moreover, the number of GFP+ EVs after 1 month is higher in EV-GuardTM buffer compared to those in PBS, as analyzed by fluorescent NTA (Figure 3 box).

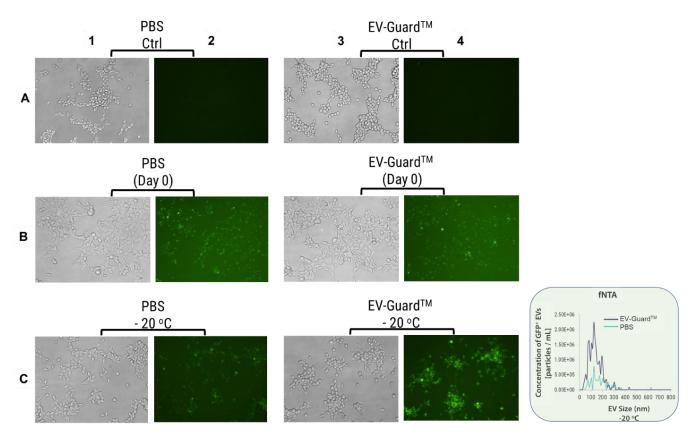


Figure 3. GFP-loaded EVs were stored in either PBS (columns 1 and 2) or EV-Guard[™] (columns 3 and 4) at -20°C for 1 month before being introduced to HEK293 cells for transfection (Row C). Row A represents the negative control (no GFP EV), while Row B is the positive control (no storage). After 1 month at -20°C, the number of GFP+ EVs was analyzed using fluorescent NTA (as shown in the box to the right).

Related Products

Exosome/EV Isolation					
High-purity, high-yield SEC-based isolation from a range of biofluids					
High-throughput SEC-based isolation from serum and plasma, 96-well format	SmartSEC™ HT	SSEC096A-1			
Single format SEC-based isolation, validated for human serum, plasma, and CSF	SmartSEC™ Single	SSEC200A-1			
High purity, polymer-based EV isolation					
Isolation from serum and plasma	ExoQuick® ULTRA	EQULTRA-20A-1			
Isolation from tissue culture media and other fluids	ExoQuick-TC® ULTRA	EQULTRA-20TC-1			
General p	urpose, polymer-based EV isolation				
Isolation from serum and plasma	ExoQuick®	EXOQ20A-1			
Isolation from tissue culture media and other fluids	ExoQuick-TC®	EXOTC50A-1			
Protein Characterization of Exosomes					
Antibody Arrays	Exo-Check Exosome Antibody Array	EXORAY200B-4 EXORAY210B-8			
ELISA	ExoELISA-ULTRA Complete Kits	EXEL-ULTRA-CD63-1 EXEL-ULTRA-CD81-1 EXEL-ULTRA-CD9-1			
RN	A extraction from Exosomes				
Obtain high yields of total exosome/EV RNA, including small RNAs	EVery EV RNA Isolation Kit	EVery100B-1			
Flexible & efficient RNA extraction from exosomes	SeraMir Exosome RNA Column Purification Kit	RA808A-1			
cDNA synthesis from EV RNA					
Quick and easy cDNA synthesis optimized for use with the EVery EV RNA Purification System	EVery cDNA Synthesis Kit	EVery200B-1			
EV miRNA profiling					
qPCR-based exosomal miRNA profiling plate for human serum and plasma samples	EVery miRNome Profiler for Human Serum and Plasma	EVery500B-1			
Synthetic small RNA controls for your RNA isolation and cDNA synthesis steps	EVery miRNA Spike-in Kit	EVery600B-1			

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:

System Biosciences (SBI) 2438 Embarcadero Way Palo Alto, CA 94303

Phone: (650) 968-2200

(888) 266-5066 (Toll Free)

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 the Exo-Check Exosome Antibody Arrays (*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.

© 2023 System Biosciences (SBI), All Rights Reserved



System Biosciences (SBI) 2438 Embarcadero Way Palo Alto, CA 94303

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