

ExoQuick[®]-TC UltraPure EV Isolation Kit

For Tissue Culture

Cat # EQ-TC UltraPure_S

Cat # EQ-TC UltraPure_L

User Manual

Store Kits at +4°C - +30°C upon receipt

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PRODUCT DESCRIPTION

Why should scale come at the cost of speed, purity, and yield?

With SBI's ExoQuick®-TC UltraPure, you get all three. This next-generation dual EV isolation kit combines gentle precipitation with advanced spin-column technology to deliver maximum yields of pure, apoB-free, intact EVs in just 20 minutes - no ultracentrifuge or multiple fractionations required. The best part is that it can be scaled up to 2 liter of cell culture media (for more details, please refer to Cat# EQ-TC UltraPure_L for large-volume EV isolation). Compared to size-exclusion chromatography (SEC) and other polymer-based methods, ExoQuick®-TC UltraPure (or EQ-TC UltraPure) achieves lower albumin and IgGs carry-over and higher purity, making it the smarter choice for reliable EV isolation.

- **>90% EV Recovery** — dual-action precipitation and column purification ensures maximum EV capture from every reaction
- **Near-Complete Lipoprotein Depletion** — ApoB ELISA confirms near-undetectable lipoprotein levels, eliminating the most common confounder in EV RNA analysis and sequencing
- **Scalable from 10 mL to 2 L** — process standard flask volumes or large bioreactor harvests in a single reaction; no pre-concentration, no ultrafiltration, no pooling
- **Minimal Albumin and IgG Carry-Over** — Western blot and Ponceau S staining confirm cleaner preparations than SEC and membrane-based method
- **Consistent and Reproducible** — unlike ultracentrifugation, ExoQuick®-TC UltraPure delivers highly reproducible results across experiments without specialized equipment
- **Fast, Simple Workflow** — add ExoQuick®-TC to conditioned media, mix, centrifuge, transfer to column, brief incubation, quick spin — done in under 20 minutes of hands-on time

ExoQuick®-TC UltraPure Delivers > 50x Higher EV Yield vs. SEC

EVs were isolated from HEK293 cell culture medium using ExoQuick®-TC Ultra, ExoQuick®-TC UltraPure, size-exclusion chromatography (SEC)-based qEV, ultracentrifugation, and tangential flow filtration (TFF). EV particle numbers from each method were quantified by NTA. NTA confirms that ExoQuick®-TC UltraPure delivers superior EV yield per 10 mL of input culture media (Fig. 1A),

as well as superior purity per mg of EV protein equivalent (Fig. 1B).

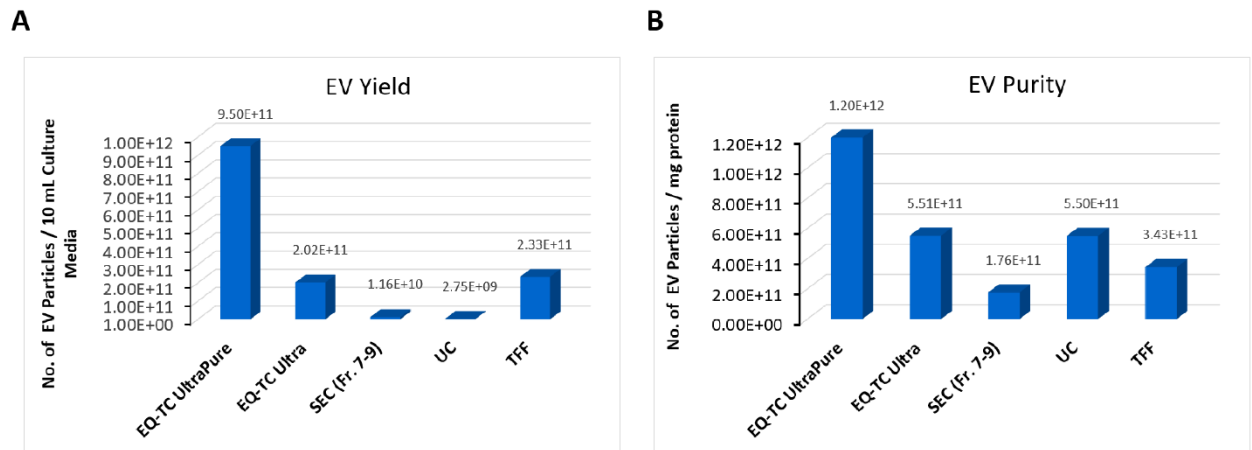


FIGURE 1. EXOQUICK®-TC ULTRAPURE PROVIDES HIGHER EV YIELD AND PURITY COMPARED TO OTHER ISOLATION METHODS.

A SIMPLE WORKFLOW DIAGRAM

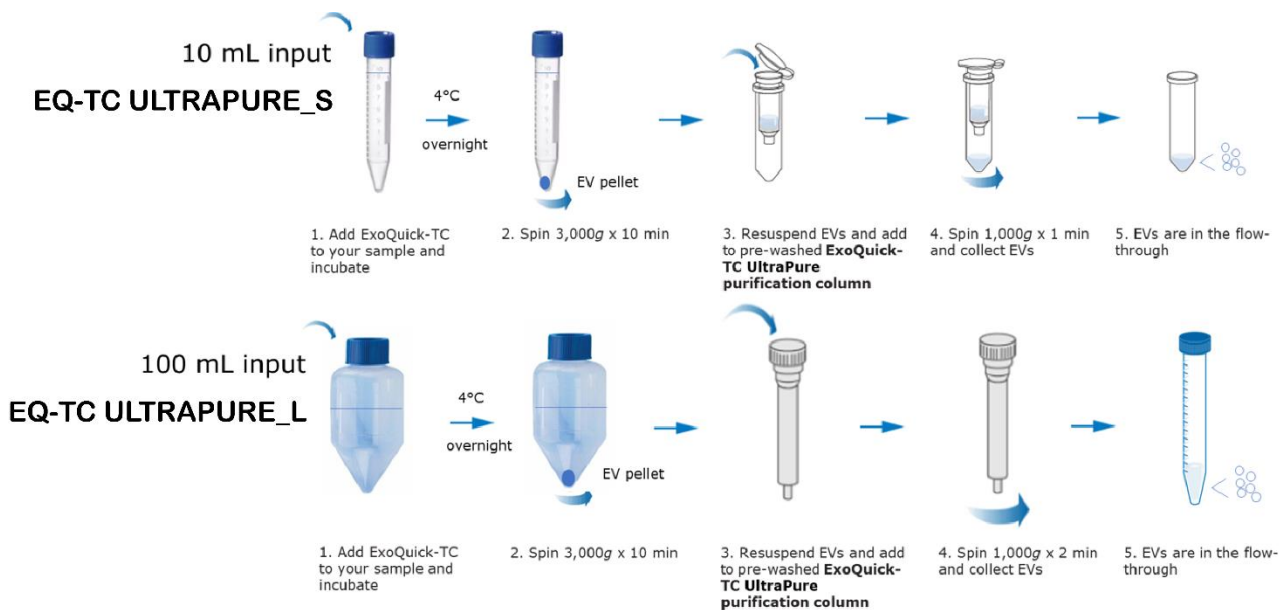


FIGURE 2. EXOQUICK®-TC ULTRAPURE WORKFLOW FOR EV ISOLATION FROM 10 mL and 100 mL SAMPLES.

KIT CONTENTS

EQ-TC UltraPure_S

Component	Volume / Qty.	Storage Temperature
ExoQuick®-TC	20 mL, Sterile	+4°C to +30°C
UltraPure Purification Column	10 columns	
Column Buffer	20 mL	
Collection Tubes	10	
2 mL Eppendorf Tubes	10	

***1 reaction is defined as 10 mL of cell culture medium precipitated using ExoQuick®-TC.**

EQ-TC UltraPure_L

Component	Volume / Qty.	Storage Temperature
ExoQuick®-TC	200 mL, Sterile	+4°C to +30°C
UltraPure Purification Column	10 columns	
Column Buffer	200 mL	

***1 reaction is defined as 100 ml of cell culture medium precipitated using ExoQuick®-TC.**

**** Please note that tubes for centrifugation and EV elution are not included with the EQ-TC UltraPure_L kit.**

The kit is shipped at +4°C to +30°C and should be stored at +4°C - +30°C. Properly stored kits are stable for 12 months from the date received.

Component not included in the kit: Sterile 1xPBS

EQ-TC UltraPure S KIT

PROTOCOL

A. ExoQuick-TC Precipitation

1. Collect the cell culture medium and centrifuge it at $1,200 \times g$ at room temperature for 5 minutes to remove any cells.
2. Transfer the supernatant to a new tube and centrifuge at $3,000 \times g$ for 15 minutes to remove any cellular debris.

! **OPTIONAL:** To remove large vesicles, centrifuging at $10,000\text{-}12,000 \times g$ for 15 minutes, or filtering the sample containing the EVs through a 0.2-micron pore size filter.

3. Transfer the supernatant to a new tube.
4. Add the appropriate volume of ExoQuick-TC to the clarified cell culture medium, as shown in the table.

Biofluid	Sample Volume	ExoQuick Volume	Incubation Time
Cell Culture Medium	10 ml	2 ml	O/N at 4°C

5. Mix well by inverting or shaking the tube, and incubate at 4°C overnight (O/N) or for 12 hrs. The tubes do not need to be rotated during the incubation period.

NOTE: To achieve a higher yield of EVs, overnight incubation at 4°C is always recommended.

6. Centrifuge the ExoQuick-TC/cell culture medium mixture at $3,000 \times g$ for 10 minutes. Centrifugation may be performed at either room temperature or 4°C with similar results. After centrifugation, the EVs may appear as a beige or white pellet at the bottom of the tube. For serum-free cell culture medium, an obvious pellet may not be visible. Please proceed with the protocol regardless.
7. Carefully aspirate off the supernatant. Spin down any residual supernatant and remove all traces of fluid by aspiration, taking great care not to disturb the precipitated EVs in the pellet. For serum free cell culture medium, if no pellet is observed, please leave small amount of fluid at the bottom of the tube.
8. The EV pellet may be sticky and difficult to resuspend. Please carefully and thoroughly resuspend the pellet in 400-500 μL of sterile $1\times$ PBS.

B. Purification of Isolated EVs

1. Take the **UltraPure purification column** (resin suspended in storage buffer) and remove both the top cap and the bottom closure.

! CAUTION: save the cap and the bottom closure for later steps.

2. Place the column into an empty **collection tube** and loosely screw the cap onto the top of the column.
3. Centrifuge at 1000 x g for 1 min to remove the storage buffer.
4. Discard the flow-through, then place the column back into the collection tube.
5. To wash the column, remove the cap and apply 500 µl of **Column buffer** onto the resin. Loosely screw the cap back onto the top of the column.
6. Centrifuge at 1,000 x g for 1 min. Discard the flow-through.
7. Repeat the wash step once more.
8. Discard the collection tube.
9. The column is now ready for sample loading.
10. Firmly reattach the white bottom closure to the bottom of the column.
11. Add the entire EV sample from step A-8 to the resin. Secure the screw cap **tightly** on top of the column.
12. Mix EV sample thoroughly with the resin by inverting and flicking the column at room temperature (RT). Then place the column on a rotating shaker for 5-7 minutes.

C. Sample Elution

1. Prepare a **2ml Eppendorf tube** to collect the EV sample.
 2. Remove the bottom closure, immediately place the column into the **2ml Eppendorf tube**, and loosen the cap halfway.
- ! CAUTION:** Once the bottom closure is removed, the sample will begin to flow out of the column. We strongly recommend keeping the cap loosely inserted, as residual liquid may still be present in the cap.
3. Centrifuge at 1000 x g for 1 min to collect the EVs.
 4. Isolated EV storage condition:
 - a) **For immediate use:** Store at +4°C for up to one week
 - b) **For long-term storage:** Store at -20°C or -80°C

Please Note: To preserve structural and functional integrity, we recommend adding 1/10 volume of [EV-Guard™ EV Storage Buffer – 10X concentrated \(EXSBA-10\)](#) for long term storage.

EQ-TC UltraPure_L KIT

PROTOCOL

A. ExoQuick-TC Precipitation

1. Collect the cell culture medium and centrifuge it at $1,200 \times g$ at room temperature for 5 minutes to remove any cells.
2. Transfer the supernatant to a new tube and centrifuge at $3,000 \times g$ for 15 minutes to remove any cellular debris.

! OPTIONAL: To remove large vesicles, centrifuging at $10,000\text{--}12,000 \times g$ for 15 minutes, or filtering the sample containing the EVs through a 0.2-micron pore size filter.

3. Transfer the supernatant to a new tube.
4. Add the appropriate volume of ExoQuick-TC to the clarified cell culture medium, as shown in the table.

Biofluid	Sample Volume	ExoQuick Volume	Incubation Time
Cell Culture Medium	100 ml	20 ml	O/N at 4°C

5. Mix well by inverting or shaking the tube, and incubate at 4°C overnight (O/N) or for 12 hrs. The tubes do not need to be rotated during the incubation period.

NOTE: To achieve a higher yield of EVs, overnight incubation at 4°C is always recommended.

6. Centrifuge the ExoQuick-TC/cell culture medium mixture at $3,000 \times g$ for 10 minutes. Centrifugation may be performed at either room temperature or 4°C with similar results. After centrifugation, the EVs may appear as a beige or white pellet at the bottom of the tube.
7. Carefully aspirate off the supernatant. Spin down any residual supernatant and remove all traces of fluid by aspiration, taking great care not to disturb the precipitated EVs in the pellet.
8. The EV pellet may be sticky and difficult to resuspend. Please carefully and thoroughly resuspend the pellet in 5–7 mL of sterile 1× PBS.

B. Purification of Isolated EVs

1. Take the **UltraPure purification column** (resin suspended in storage buffer) and remove both the top cap and the bottom closure.

! CAUTION: save the cap and the bottom closure for later steps.

2. Place the column in an empty **15 mL Corning conical tube** and loosely screw the cap onto the top of

the column.

3. Centrifuge at 1000 x g for 1 min to remove the storage buffer.
4. Discard the flow-through, then place the column back into the collection tube.
5. To wash the column, remove the cap and apply 5 ml of **Column buffer** onto the resin. Loosely screw the cap back onto the top of the column.
6. Centrifuge at 1,000 x g for 1 min. Discard the flow-through.
7. Repeat the wash step once more.
8. Discard the collection tube.
9. The column is now ready for sample loading.
10. Firmly reattach the bottom closure to the bottom of the column.
11. Add the entire EV sample from step A-8 to the resin. Secure the screw cap **tightly** on top of the column.
12. Mix EV sample thoroughly with the resin by inverting and flicking the column at room temperature (RT). Then place the column on a rotating shaker for 5-7 minutes.

C. Sample Elution

1. Have a new 15 mL Corning tube ready as the **sample collection tube** for collecting EVs.
 2. Remove the bottom closure, immediately place the column into the **sample collection tube**, and loosen the cap halfway.
- ! CAUTION:** Once the bottom closure is removed, the sample will begin to flow out of the column. We strongly recommend keeping the cap loosely inserted, as residual liquid may still be present in the cap.
3. Centrifuge at 1000 x g for 2 min to collect the EVs.
 4. Isolated EV storage condition:
 - a) **For immediate use:** Store at +4°C for up to one week
 - b) **For long-term storage:** Store at -20°C or -80°C

Please Note: To preserve structural and functional integrity, we recommend adding 1/10 volume of [EV-Guard™ EV Storage Buffer – 10X concentrated \(EXSBA-10\)](#) for long term storage.

EXAMPLE DATA AND APPLICATIONS

ExoQuick®-TC UltraPure Delivers Cleaner EV, Near-Complete Lipoprotein Removal

Culture medium (10 mL) from the XPACK530 HEK293 stable cell line – containing copGFP-loaded EVs – was used for isolation. Western blot analysis shows that EVs isolated with ExoQuick-TC UltraPure contain higher levels of EV-specific markers and exhibit minimal albumin carryover compared to the SEC-based qEV method, indicating both higher yield and superior purity (Fig. 3A). EV integrity is also well preserved, as indicated by robust copGFP expression. ApoB ELISA analysis reveals that EVs isolated using ExoQuick-TC UltraPure contain nearly undetectable ApoB levels, confirming minimal lipoprotein contamination (Fig. 3B).

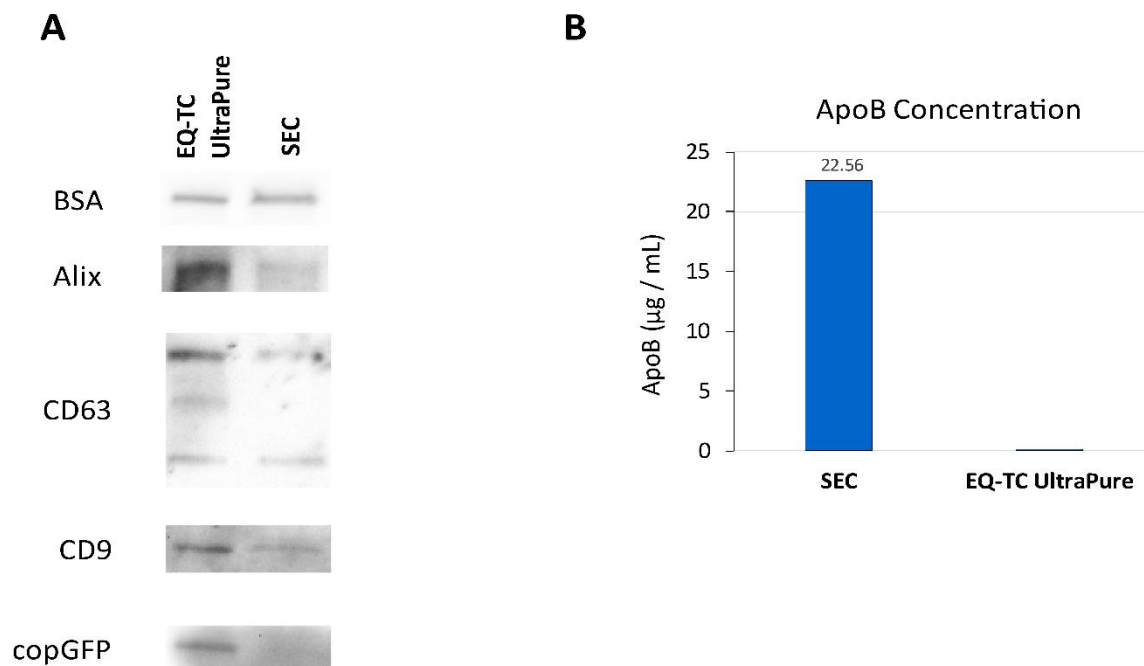


FIGURE 3. EXOQUICK®-TC ULTRAPURE DELIVERS HIGHER YIELD AND SUPERIOR PURITY COMPARED TO SEC-BASED qEV.

ExoQuick®-TC UltraPure vs. Membrane-Based Method

For this experiment, 100 mL of culture medium from an XPACK530 stable cell line - containing copGFP-loaded EVs - was processed as one reaction. Ponceau S staining confirms that ExoQuick-TC UltraPure preparations are significantly cleaner compared to tangential flow filtration (TFF; Fig. 4A). Western blot analysis demonstrates higher EV yield, strong EV marker expression, greater purity with minimal albumin carryover, and improved vesicle integrity indicated by copGFP expression, all compared to TFF (Fig. 4B).

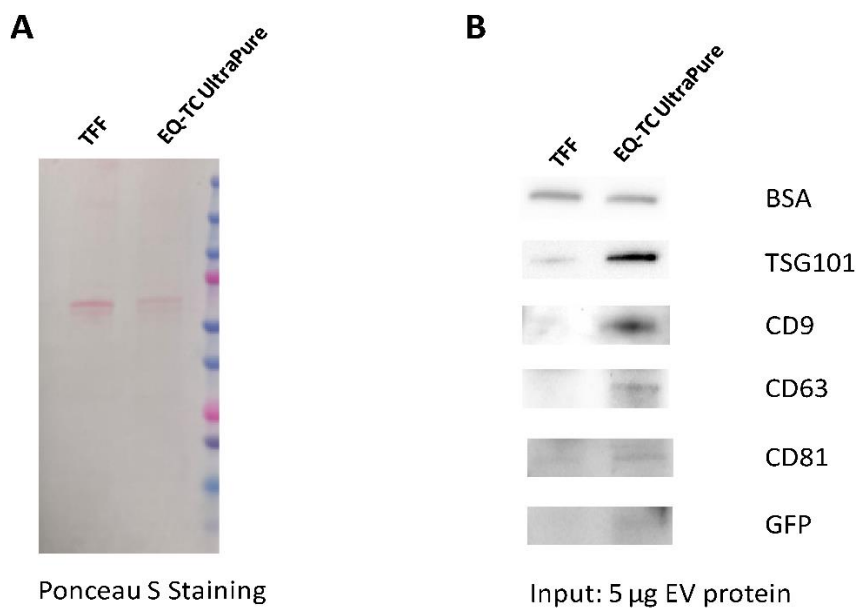


FIGURE 4. EXOQUICK®-TC ULTRAPURE IS SUITABLE FOR LARGE-SCALE EV ISOLATION WHILE MAINTAINING SUPERIOR QUALITY.

ExoQuick®-TC UltraPure Retains Morphological Integrity of Isolated EVs

Transmission electron micrographs of EVs isolated from HEK 293 cell culture medium using ExoQuick®-TC UltraPure. The same sample is shown at two different magnifications. Multiple vesicles with typical EV morphology are visible in each image (Fig. 5).

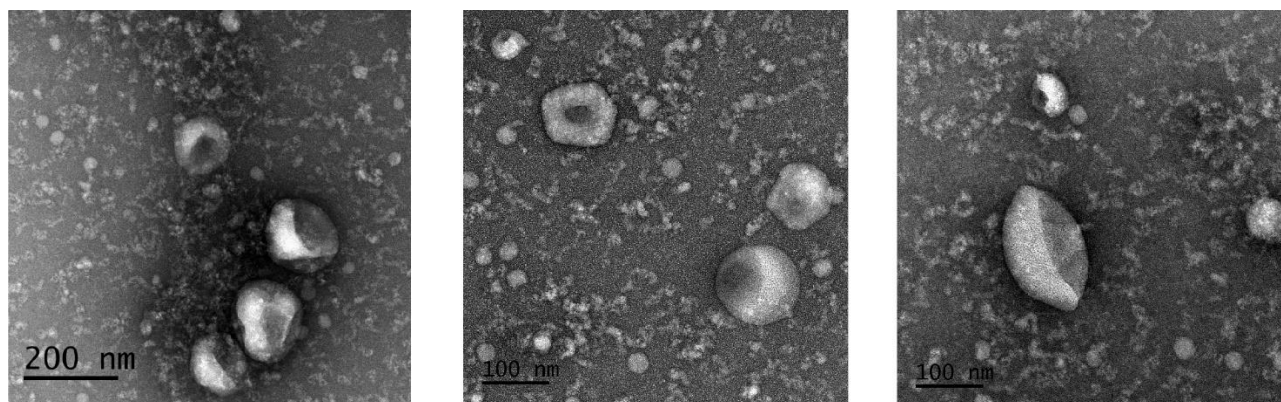


FIGURE 5. EVs ISOLATED USING EXOQUICK®-TC ULTRAPURE DISPLAY TYPICAL NATIVE EV MORPHOLOGY.

TECHNICAL SUPPORT

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

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LICENSING AND WARRANTY STATEMENT

Limited Use License

Use of ExoQuick®-TC UltraPure for EV Isolation (*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 following 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.

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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 at SBI's sole discretion. This limited warranty shall not be extended 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.

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