USER MANUAL

SMARTSEC™-TC EV ISOLATION KIT

FOR TISSUE CULTURE MEDIA

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

Cat # SSEC-TC-200A-1

System Biosciences

FUELING YOUR INNOVATION

Version 1.1
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CONTENTS

PRODUCT DESCRIPTION

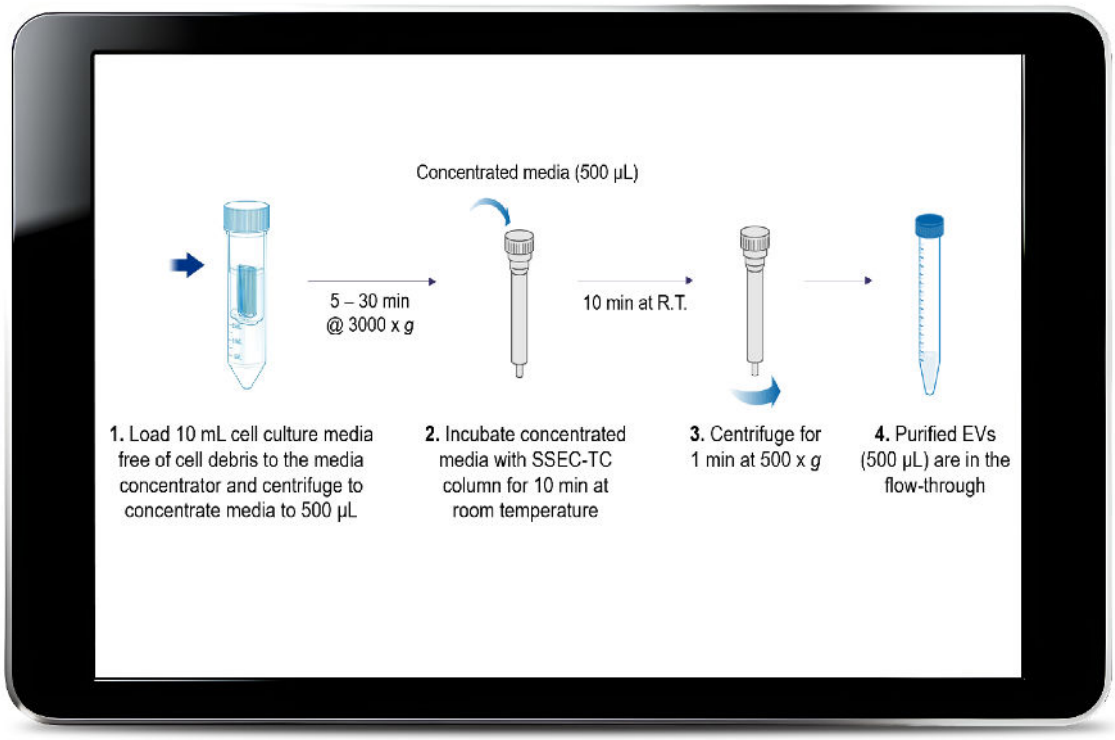
Get outstanding SEC-based EV isolation from tissue culture media with SmartSEC™ -TC

SmartSEC-TC uses the same proprietary chromatography-based extracellular vesicle (EV) isolation technology as our popular SmartSEC Single, SmartSEC HT plate and SmartSEC Mini kit, and is now optimized for use with tissue culture media. SmartSEC technology combines all the benefits of size exclusion chromatography (SEC) - purity, yield, reproducibility, and preservation of EV integrity - with an advanced contaminant-trapping technology. The new SmartSEC-TC (SSEC-TC) kit incorporates an initial step to concentrate large volumes of tissue culture media, allowing EV-containing samples to be reduced to a convenient volume for downstream processing. The result is best-in-class EV isolation from culture media that is fast, easy, and clean.

- New generation of SmartSEC™ technology designed for tissue culture media
- Mixed-mode chromatography – no need to collect multiple fractions, only one single fraction is collected
- Fast and easy isolation of high yield and high purity EVs
- Native process - no addition of precipitating reagents required
- Higher EV concentration and easier workflow compared to competitors' conventional size exclusion chromatography kits
- Compatible with a wide range of downstream applications, including Western blotting, nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), RNA-sequencing (NGS), proteomics and lipidomic profiling

The SmartSEC-TC workflow can be completed in as little as 1 hour with less than 10 minutes of hands-on time.

Simply apply 10 mL of tissue culture media (free of cellular debris) to the media concentrator, centrifuge to concentrate the media to 500 µL, directly apply it to the pre-washed SSEC-TC column, incubate, and centrifuge to elute the EVs.



THE SIMPLE SMARTSEC-TC WORKFLOW

KIT CONTENTS

Component	Qty/Volume	Storage Temperature
Media Concentrator	8 concentrators	+4°C - +30°C
SmartSEC-TC column	8 columns	
Column buffer	50 mL	
Column closures	8	

The kit is shipped at +4°C - +30°C and should be stored at +4°C - +30°C. Properly stored kits are stable for 9 months from the date received.

PROTOCOL

The protocol is optimized for 10 mL of tissue culture media samples. We recommend culturing cells in exosome depleted FBS, such as SBI's Exo-FBS, or under low-serum conditions for high purity EV isolations.

If your starting volume of culture media exceeds 15 mL, simply perform additional SSEC-TC reactions for EV isolation.

Sample preparation and concentration:

1. Collect the tissue culture media sample and centrifuge at 3,000 × g for 15 minutes to remove cellular debris.
2. Apply 10 mL of the pre-cleared media without cellular debris from step 1 to the filter device part of the media concentrator.
3. Centrifuge at 3,000 × g for 5-30 minutes to concentrate the media.

   ! CAUTION: Check the concentrated volume in the media concentrator every 5-10 minutes. Adjust the time if needed to obtain a desired volume (300 µL - 600 µL) of concentrated media. The required time varies depending on the type of culture media. Serum-free samples typically require 5 minutes or less, while regular media or samples with a higher concentration of EVs and/or proteins will take longer to concentrate.
4. Transfer the final concentrated volume of sample (~ 500 µL) into a clean Eppendorf tube.
5. Centrifuge at **10,000 x g for 10 minutes** to remove any debris.
6. The concentrated media sample is now ready for EV isolation. Place the sample in an ice bath until the next step.

EVs isolation step:

7. Remove the cap and twist off the bottom closure from the **SmartSEC-TC column** (resin is contained in storage buffer).
8. Place the column in an empty **Collection tube** (15 mL conical tube, not provided).
9. Centrifuge at **500 x g for 1 min** to remove the storage buffer.
10. Add 1 mL of **Column buffer** to the column.
11. Centrifuge at **500 x g for 1 min** to wash the column.
12. Repeat Steps 10 and 11 one more time for a total of 2 washes.
13. Discard the collection tube. The column is now ready for use.
14. Place a **column closure** onto the bottom of column and apply your sample to the top of the resin bed. Avoid transferring the cell debris pellet at the bottom of the tube.
15. Add an additional amount of column buffer to the column to bring the total sample volume up to **500 µL** (For 500 µL of starting sample applied, no additional buffer is needed). Replace and tighten

the cap to close the column. Gently invert the column 5 times to ensure the sample is well mixed with resin.

16. Incubate at RT for **10 min with continuous mixing by gentle rotation**.
17. Have a sample collection tube of your choice ready for EV collection (not provided).
18. Loosen the cap halfway and remove the bottom closure.

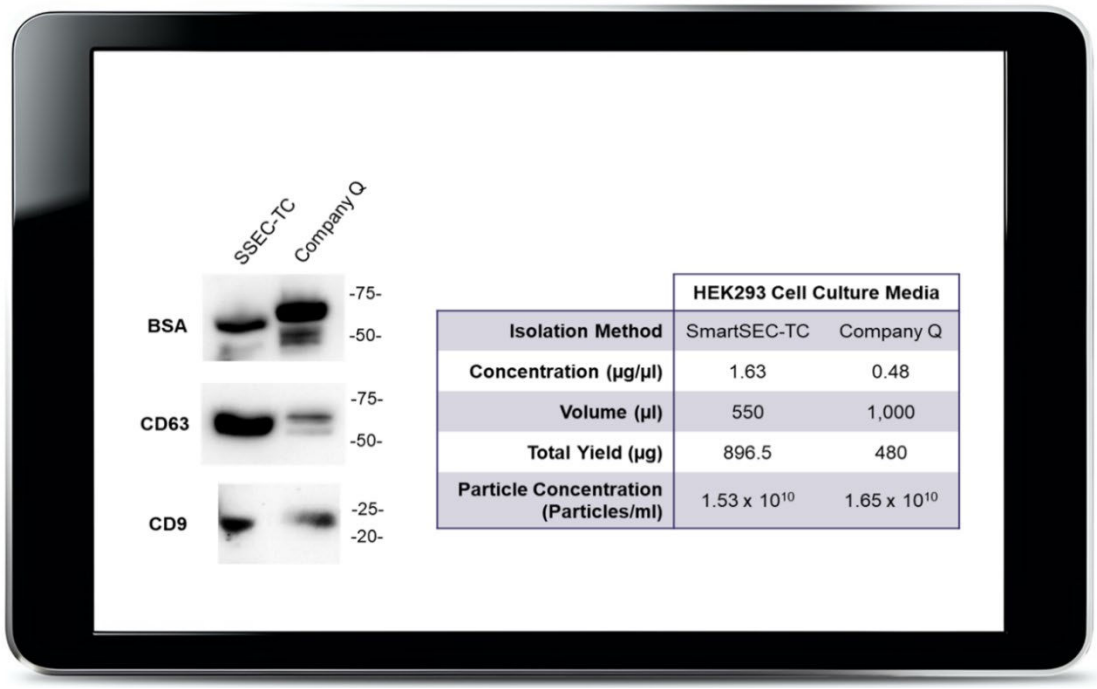
    ! CAUTION: We strongly recommend keeping the cap on the column (still loosened halfway) due to the residual liquid that might still be present in the cap.
19. Immediately place the column in the sample collection tube (step 17) and centrifuge at **500 x g for 1 min** to collect the EVs.
20. Store the isolated EVs:
    a) For immediate use: at +4°C for up to one week

    b) For long-term storage: at -20°C or -80°C
21. **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

SmartSEC-TC outperforms Company Q's SEC technology

To evaluate how well SmartSEC-TC performs compared to a competitor's SEC-based columns, EVs were isolated from 10 mL of HEK293 cell culture media (containing 10% exosome depleted FBS; Exo-FBS) using both SmartSEC-TC and Company Q's columns. Western blot and fluorescent nanoparticle tracking analysis (fNTA) was performed on 5 µg of protein equivalent from each isolation method (Figure 1). EVs isolated using SmartSEC-TC showed comparable particle concentrations but significantly higher levels of EV marker proteins (CD63 and CD9) and lower carry-over proteins, such as BSA from the FBS in the cell culture media (Figure 1). In contrast, EVs isolated from multiple pooled fractions using the column from Company Q exhibited lower purity. In addition, SmartSEC-TC achieved EV isolation at ~3-fold higher protein equivalent concentration compared to Company Q's SEC column (Figure 1), demonstrating its superior performance overall.



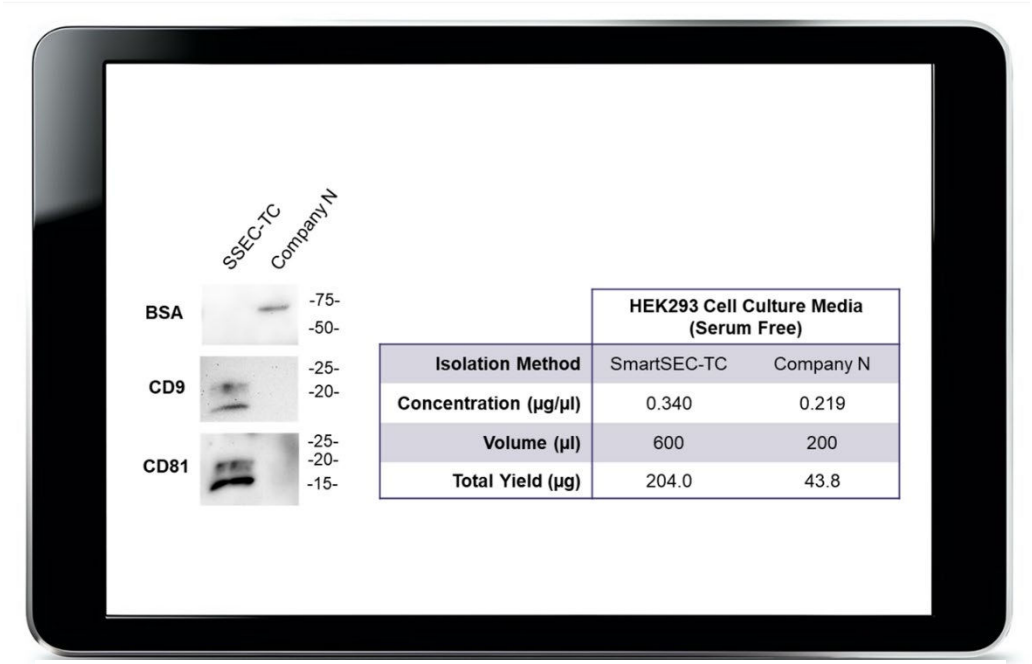
	HEK293 Cell Culture Media	
Isolation Method	SmartSEC-TC	Company Q
Concentration (µg/µl)	1.63	0.48
Volume (µl)	550	1,000
Total Yield (µg)	896.5	480
Particle Concentration (Particles/ml)	1.53×10^{10}	1.65×10^{10}

FIGURE 1. SMARTSEC-TC DELIVERS HIGHER YIELDS OF CLEANER EVs THAN MULTIPLE POOLED FRACTIONS FROM COMPETITOR COMPANY Q'S SEC COLUMN.

SmartSEC-TC is suitable for isolating EVs from serum free cell culture media

For high purity EV isolations, it can be crucial to culture the cells under serum free condition, but the yield of EVs may be significantly compromised due to the harsh growth condition without serum. SmartSEC-TC can effectively isolate EVs from serum free cell culture media with high yield and purity. EVs were isolated from 10 mL of serum free HEK293 cell conditioned media after 4 days in culture, using both SmartSEC-TC and a competitor Company N's bead-based EV isolation method. EV marker proteins were analyzed by

Western blot from each isolation method (Figure 2) and particle counts were measured with fNTA (Figure 3). EVs isolated using SmartSEC-TC shows significantly higher EV marker proteins (CD9 and CD81) with minimal BSA carry-over in the cell culture media compared to EVs isolated using Company N's method (Figure 2). SmartSEC-TC isolated EVs also have over 6-fold higher particle concentrations and over 3-fold higher purity than Company N isolated EVs (Figure 3), demonstrating the effectiveness of SmartSEC-TC in isolating EVs from serum free cell culture media with high yield and purity.



	HEK293 Cell Culture Media (Serum Free)	
Isolation Method	SmartSEC-TC	Company N
Concentration (µg/µl)	0.340	0.219
Volume (µl)	600	200
Total Yield (µg)	204.0	43.8

FIGURE 2. WESTERN BLOT ANALYSIS SHOWS THAT SMARTSEC-TC DELIVERS MUCH HIGHER YIELD AND PURITY OF EVs FROM SERUM FREE CELL CULTURE MEDIA THAN COMPANY N'S METHOD. 5 µg OF PROTEIN EQUIVALENT OF EVs WERE LOADED PER LANE.

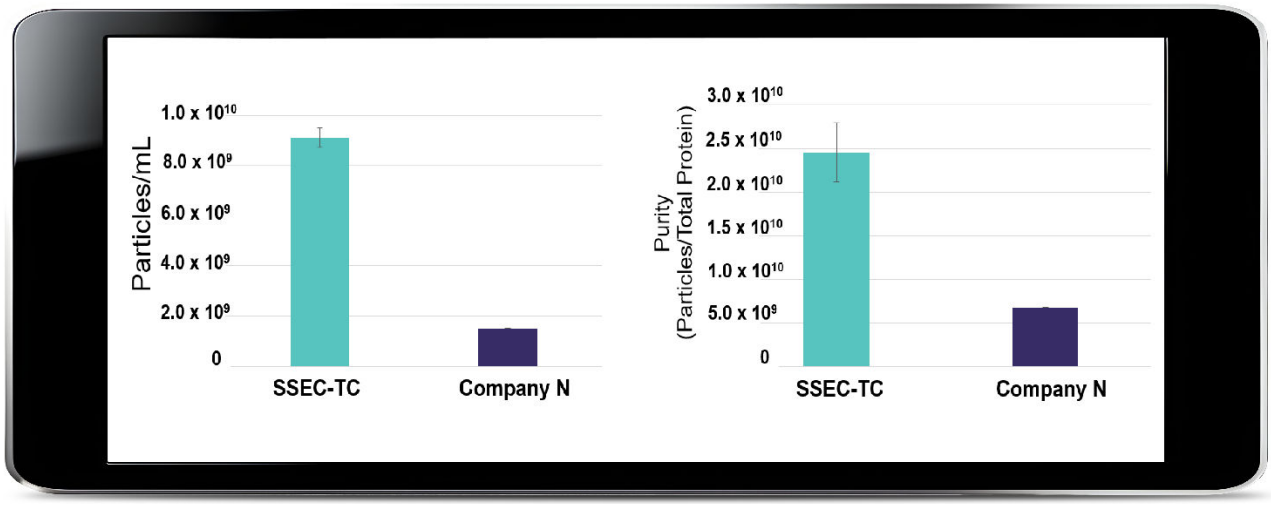
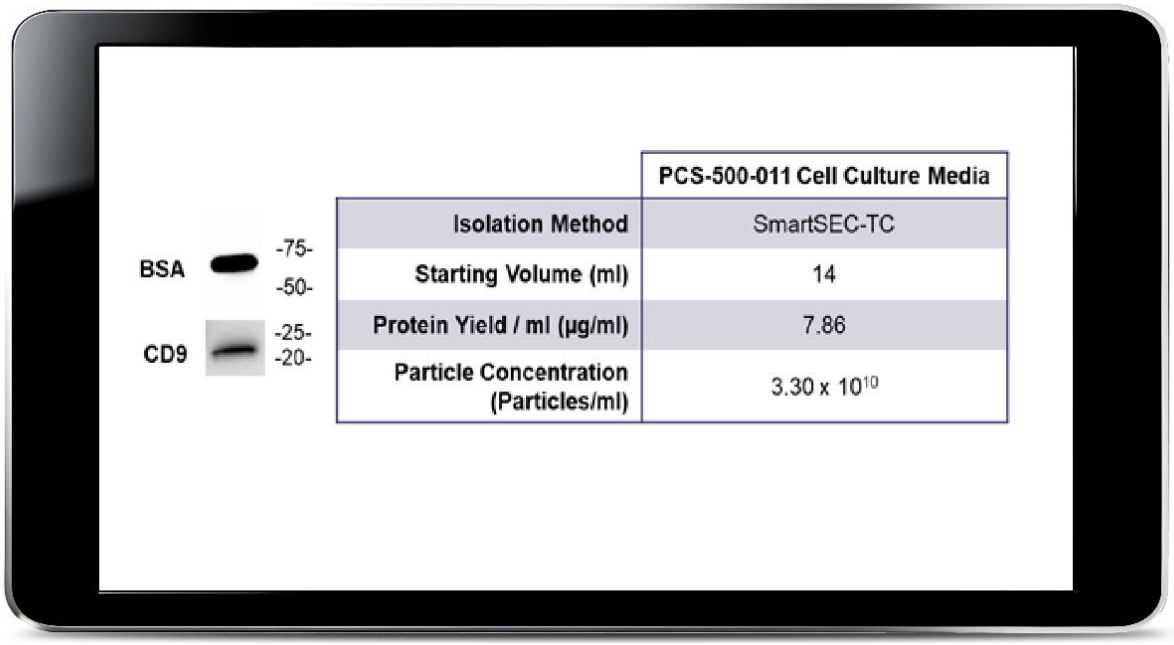


FIGURE 3. fNTA SHOWS THAT SMARTSEC-TC CAN DELIVER HIGHER YIELDS OF EV PARTICLES THAN COMPANY N FROM SERUM-FREE CELL CULTURE MEDIA.

SmartSEC-TC cleanly isolates EVs from stem cells

SmartSEC-TC can isolate EVs from stem cells cultured in media under low serum conditions. EVs isolated from PCS-500-011 human adipose-derived mesenchymal stem cell culture media (with 0.2% FBS) using SmartSEC-TC showed typical EV protein markers on western blot (Figure 4) and an excellent EV particle yield as analyzed by fNTA (Figure 4.)



	PCS-500-011 Cell Culture Media
Isolation Method	SmartSEC-TC
Starting Volume (ml)	14
Protein Yield / ml (µg/ml)	7.86
Particle Concentration (Particles/ml)	3.30×10^{10}

FIGURE 4. SMARTSEC-TC ISOLATES EVs FROM STEM CELL CULTURE MEDIA.

EVs isolated using SmartSEC-TC possess typical EV morphology

EVs isolated from HEK293 cell culture media and from PCS-500-011 human adipose-derived mesenchymal stem cell culture media using SmartSEC-TC possess typical EV morphology—intact vesicles with lipid bi-layer membranes—with little visible background debris as shown by transmission electron microscopy (TEM) (Figure 5).

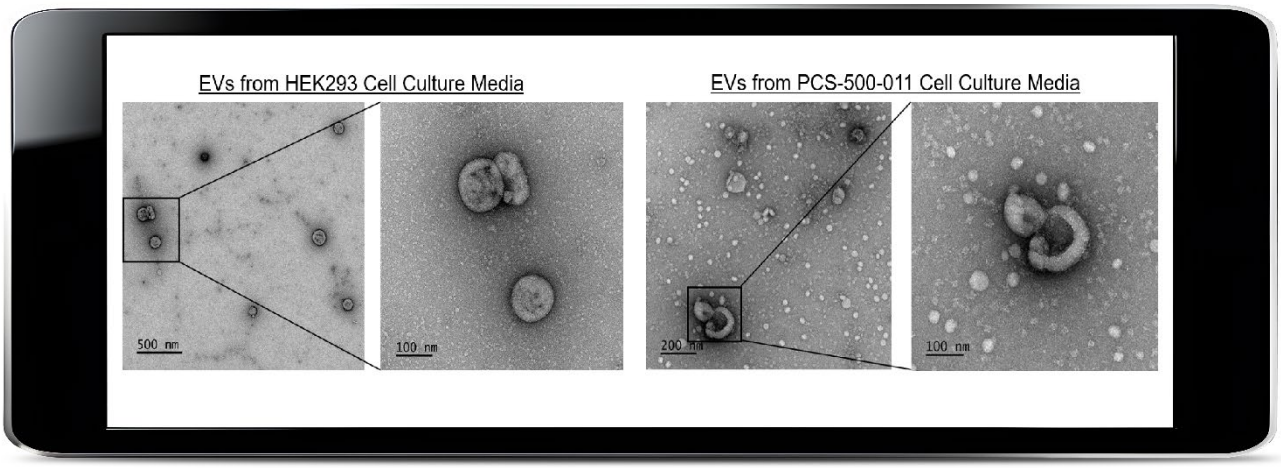


FIGURE 5. EVs ISOLATED USING SMARTSEC-TC POSSESS TYPICAL EV MORPHOLOGY.

EVs isolated by SmartSEC-TC show positive EV protein markers by ExoCheck™ assay

SmartSEC-TC isolated EVs (50 µg of protein equivalent) from HEK293 cell culture media and PCS-500-011 human adipose-derived mesenchymal stem cell culture media were analyzed using SBI's Exo-Check™ Exosome Antibody Array assay (Figure 6). The results show positive signals of EV marker proteins identified in the EVs isolated by SmartSEC-TC.

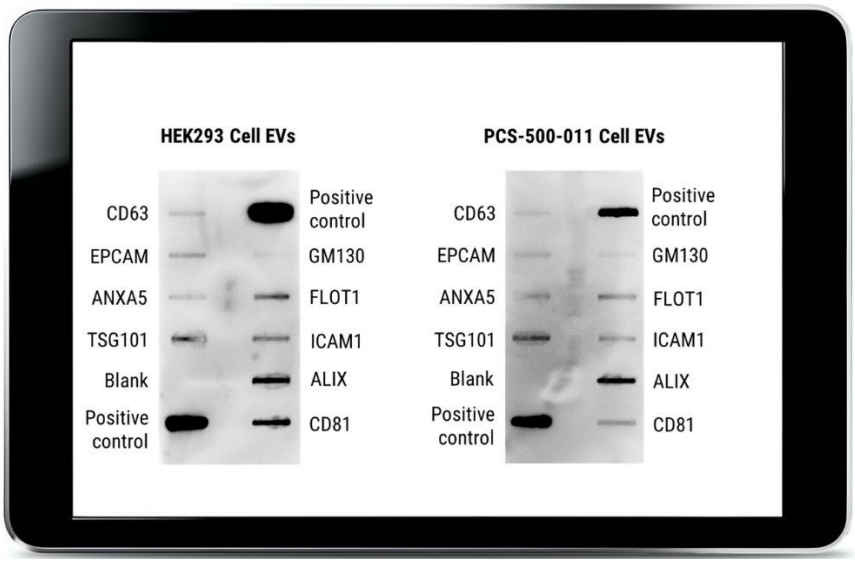


FIGURE 6. SMARTSEC-TC ISOLATES EVs WITH POSITIVE EV MARKER PROTEINS.

EVs isolated by SmartSEC-TC contain high quality RNAs

EV RNA was extracted from EVs isolated by SmartSEC-TC from Hela and HEK293 cell culture media (CCM) using SBI's EVery EV RNA Isolation Kit (EVery100B-1). The RNA samples were analyzed using an Agilent Bioanalyzer and showed excellent size distribution with typical patterns of small RNA enrichment and minimal genomic DNA contamination as evidenced by electrophoretic spectra and gel images (Figure 7).

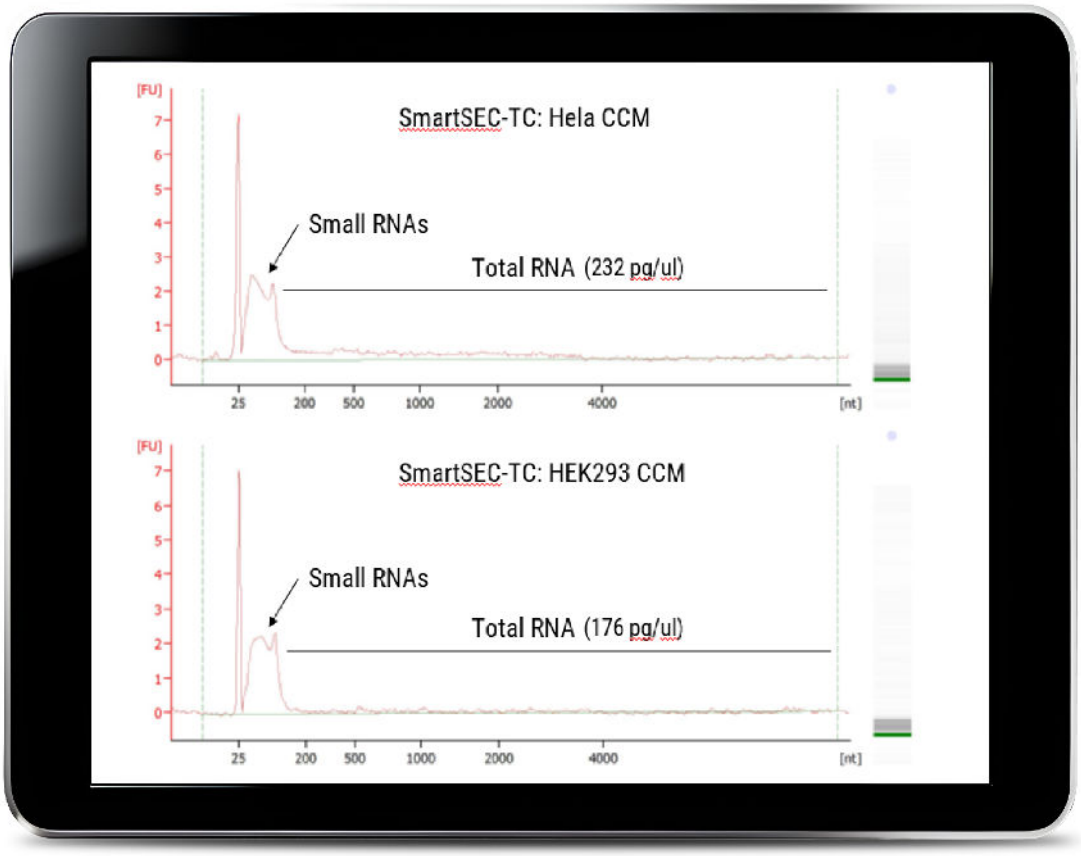


FIGURE 7. EXCELLENT SIZE DISTRIBUTION OF RNAS DERIVED FROM SMARTSEC-TC ISOLATED EVs.

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

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Use of SmartSEC-TC 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:
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- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

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