XStamp Pro

Cat # XSTP900A-1, XSTP905A-1, XSTP910A-1, XSTP915A-1

User Manual

Storage: Store at -80°C

Version 1
9/16/2019

Contents

Product Description

Creating target-specific EVs just got a whole lot easier

While conventional methods for creating target-specific extracellular vesicles (EVs) are highly robust (see our XStamp Cloning and Expression Lentiector, Cat# XSTP710PA-1), they involve cloning and manipulation of the producer cells which can be challenging, especially if the cells are hard to transfect. To streamline this process and help researchers more quickly and easily create EVs that preferentially localize at a specific target, SBI turned to an exciting new technology, XStamp™ Pro, which works on already purified EVs—no cloning or transfection required. Simply incubate purified EVs with the XStamp Pro ScFv or XStamp Pro Streptavidin for one-hour and then isolate your target-specific EVs.

How XStamp Pro anti-HIV (Cat# XSTP905A-1), XStamp Pro anti-CD16 (Cat# XSTP910A-1), and XStamp Pro anti-PD-1 (Cat# XSTP915A-1) work

XStamp Pro takes advantage of the same EV surface-directing protein used in our original XStamp products but skips the expression vector and the transfection step for a faster, simpler workflow. You get an already purified peptide consisting of the target-specific ScFv fused to the EV surface-directing protein—the XStamp Pro ScFv. Upon incubation with isolated EVs, the XStamp Pro ScFv self-inserts into EV membranes creating your target-specific EVs (Figure 1). Incubation is complete in 1 hour, after which the target-specific EVs can be isolated using the included ExoQuick-TC reagent or other isolation method of your choice, and then used to deliver cargo.

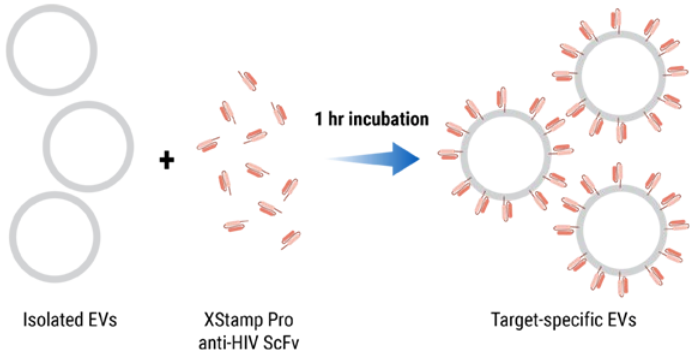


Figure 1. The workflow for XStamp Pro anti-HIV, XStamp Pro anti-CD16 and XStamp Pro anti-PD-1.

How XStamp Pro Streptavidin (Cat.# XSTP900A-1) works

XStamp Pro Streptavidin works in a similar fashion as the other XStamp Pro kits, but instead of fusing the EV surface-directing protein to an scFv, the XStamp Pro Streptavidin Kit includes an already purified peptide consisting of streptavidin fused to the EV surface-directing protein—the XStamp Pro Streptavidin. Upon incubation with isolated EVs, the XStamp Pro Streptavidin self-inserts into EV membranes creating your streptavidin-decorated EVs (Figure 2). Incubation is complete in 1 hour, after which the decorated EVs can be isolated using the included ExoQuick-TC reagent or method of your choice, and then given target specificity through the addition of a biotinylated targeting moiety, such as a biotinylated ScFv antibody. Once the targeting specificity has been programmed in, the EVs are ready to deliver cargo.

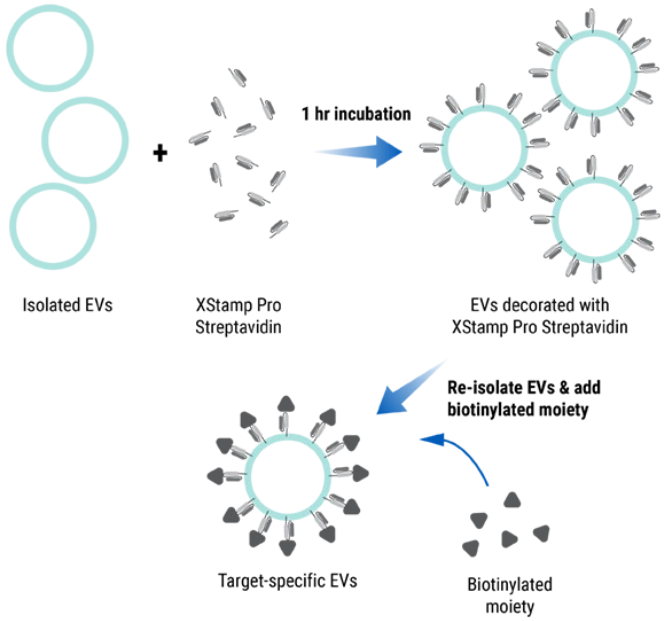


Figure 2. The workflow for XStamp Pro Streptavidin.

List of Components

Component	Qty/Volume	Storage Temperature
XStamp Pro Purified protein	100 µg	-80^{0}C
ExoQuick-TC	2 mL	RT

*The kit is for 10 individual labeling reactions

Additional required and optional equipment not included in kit

- Pipettes and tips
- Pre-isolated extracellular vesicles (EVs)
- Ultracentrifuge (*optional*; for isolating XStamp Pro-decorated EVs from the reaction)

Storage

XStamp Pro is shipped on dry ice and should be stored at -80°C on receipt. Aliquoting for single reaction use is recommended. Properly stored kits are stable for 6 months from the date received.

Protocol

1. Measure protein concentration of your EV sample

2. To decorate the EVs, add 10 µg XStamp Pro to 300 µg protein equivalent of EVs in a total of 300 µL buffer (preferably PBS)

! **Note**: Optimization of XStamp Pro amount added per reaction may be required depending on the levels and/or accessibility of the target protein on the recipient cells

3. Incubate the reaction at room temperature for 1h

4. Isolate the decorated EVs from the reaction by your preferred method, OR by precipitation using ExoQuick-TC (included) and the protocol below

Optional: ExoQuick-TC step

a. Add 100 uL of **ExoQuick-TC** reagent and mix with pipetting. **Do not vortex.**
b. Incubate it at **4°C overnight.**
c. Centrifuge the sample for 5 minutes at 14,000 rpm in a microfuge (top speed)
d. Remove the supernatant and resuspend the decorated exosome pellet in buffer of choice for downstream application.

5. The decorated EVs are now ready for delivery

Example Data and Applications

XStamp Pro Streptavidin Targeting EV Kit Data (Figures 3 and 4)

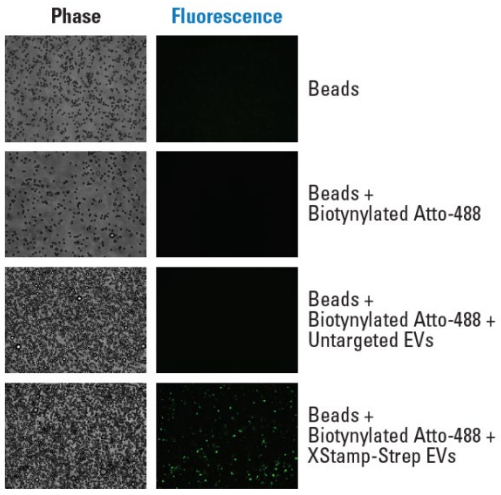


Figure 3. EVs engineered using XStamp Pro Streptavidin efficiently bind a biotinylated target. Using latex sulfide aldehyde beads which will bind EVs but not biotinylated atto-488 dye, we show that there is no fluorescence from beads alone (top row), beads with biotinylated atto-488 (second row), or beads with biotinylated atto-488 and untreated EVs (third row). Only EVs decorated with XStamp Pro Streptavidin (bottom row) can bind biotinylated atto-488 and, thus, fluoresce.

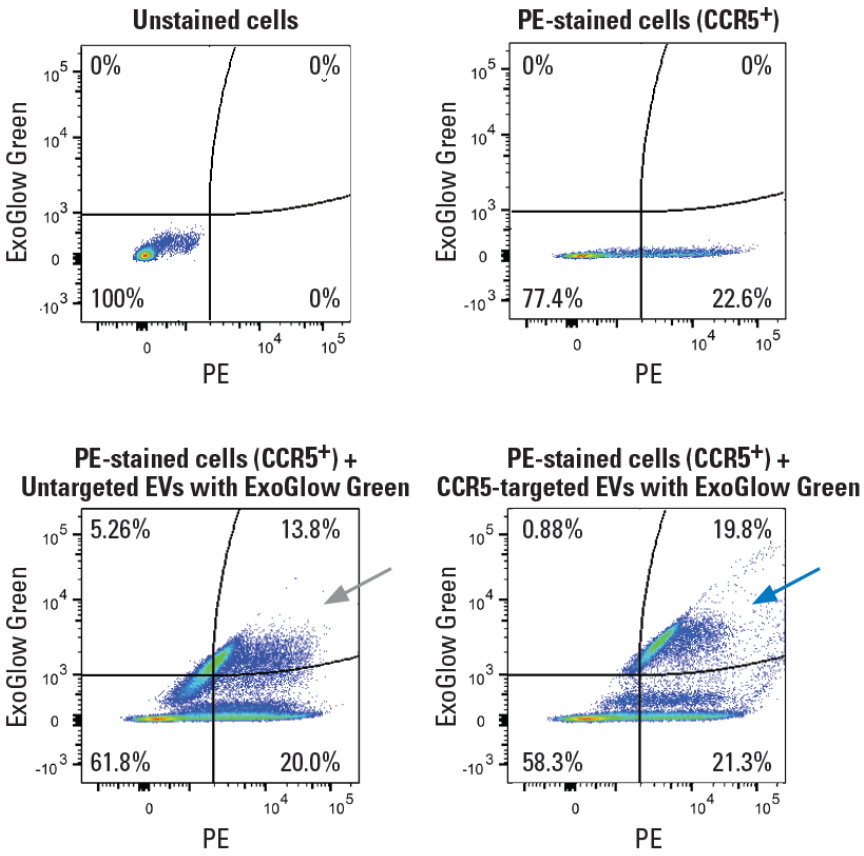


Figure 4. EVs engineered using XStamp Pro Streptavidin and biotinylated anti-CCR5 efficiently target CCR5+ cells and are internalized. We labeled serum-derived EVs with ExoGlow Green EV labeling dye (Cat.# EXOGP300A-1), treated labeled EVs with XStamp Pro Streptavidin to decorate EV surfaces with streptavidin, and then added biotinylated anti-CCR5 antibody to target the EVs to CCR5+ cells. After incubation with unactivated CD4+ T cells, which express CCR5, we stained the cells for CCR5 using phycoerythrin (PE), and examined the cells using flow cytometry. While cells treated with non-targeting ExoGlow Green-labeled EVs do take up EVs, this uptake is non-specific as it is independent of CCR5 status (bottom left plot). When cells are treated with EVs specific for CCR5, almost all of the ExoGlow Green label is restricted to CCR5+ cells (bottom right plot, top right quadrant), demonstrating the specificity and efficiency of the EV targeting. *Data courtesy of Drs. Pooja Bhardwaj and Satish Pillai, UCSF/ Vitalant Insititute.*

XStamp Pro anti-HIV Targeting EV Kit Data (Figures 5 and 6)

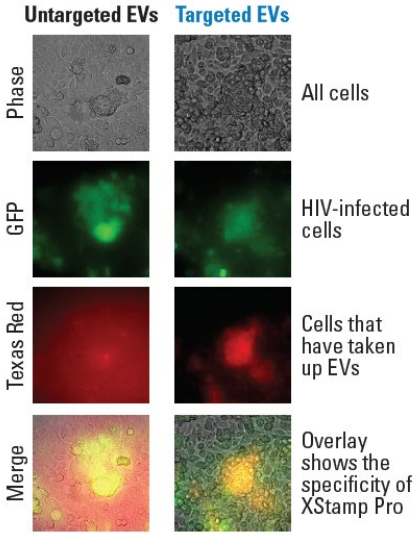


Figure 5. Target-specific EVs engineered using XStamp Pro are highly specific. We infected TZMbl cells with GFP-labeled HIV and exposed these cells to either non-targeting EVs (left column) or HIV-specific EVs coated with XStamp Pro anti-HIV ScFv and carrying a Texas Red-labeled siRNA (right column). Non-targeting EVs are not taken up by cells, and only HIV-infected cells internalize XStamp Pro anti-HIV EVs. *Data courtesy of Drs. Rafal Kaminski and Kamel Khalili, Temple University*.

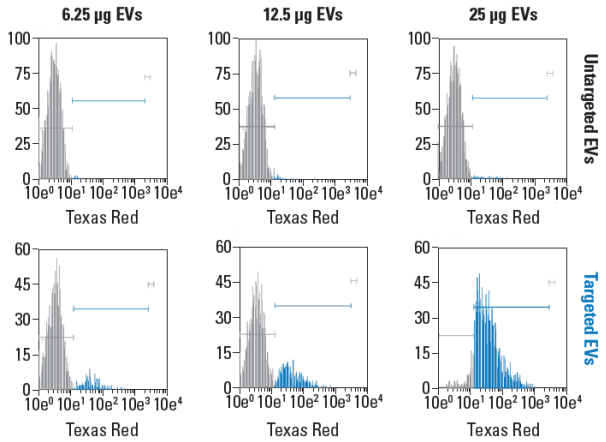


Figure 6. Targeting EVs engineered using XStamp Pro are efficiently internalized. We treated GFP-labeled Jurkat 2D10 cells (a latent NL4-3-Dgag/pol-GFP lymphoid cell line) with 6.25, 12.5, or 25 µg of XStamp Pro anti-HIV EVs carrying Texas Red-labeled siRNA . After 24 hours we assessed EV internalization using flow cytometry. Uptake of targeting EVs is efficient and dose-dependent (bottom row, Texas Red-labeled cells are shown in blue). In contrast, non-targeting EVs are not internalized, further supporting the specificity of the XStamp Pro anti-HIV targeting. *Data courtesy of Drs. Rafal Kaminski and Kamel Khalili, Temple University*.

XStamp Pro anti-CD16 Targeting EV Kit Data (Figure 7)

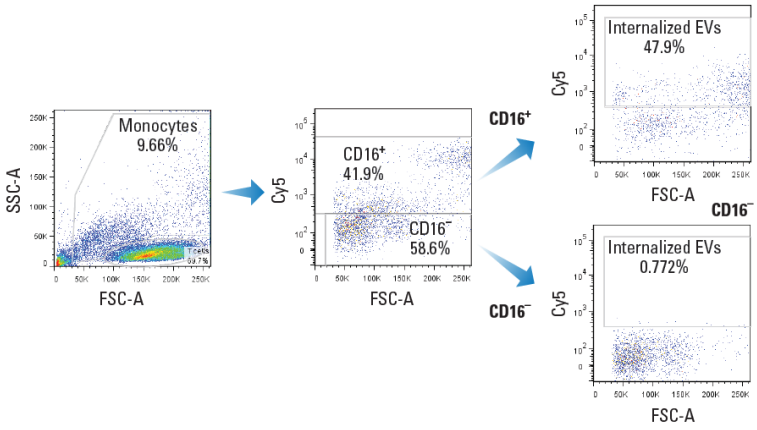
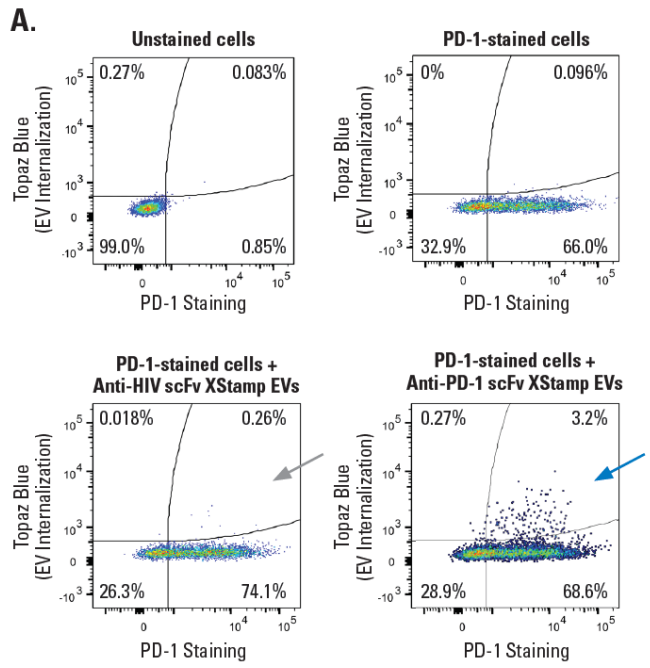


Figure 7. Target-specific EVs engineered using XStamp Pro anti-CD16 are highly specific and efficiently internalized. We stimulated human peripheral blood mononuclear cells (PBMCs) with IL-10 for 60 hours, and then added EVs coated with XStamp Pro anti-CD16 ScFv. These EVs were derived from human monocytes and loaded with a Cy5-labeled miRNA. After 15 hours, the cells were fixed, stained, and analyzed on a flow cytometer. Only CD16+ cells had internalized the EVs (47.9% of CD16+ cells compared to 0.772% of CD16− cells) demonstrating the specificity of XStamp Pro anti-CD16. *Data courtesy of Dr. Lynn Pulliam, UCSF*.

XStamp Pro anti-PD-1 Targeting EV Kit Data (Figure 8)



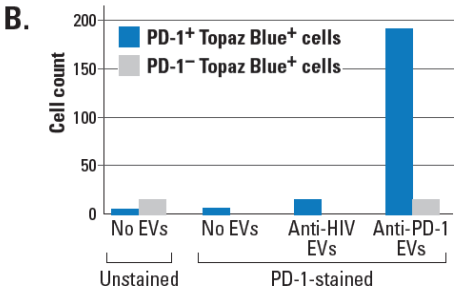


Figure 8. Target-specific EVs engineered using XStamp Pro are highly specific. (A) We labeled serum-derived EVs with SBI's ExoFlow-ONE Topaz Blue flow cytometry dye (Cat.# EXOF400A-1) and then coated them with either XStamp Pro anti-HIV ScFv (mistargeted control) or XStamp Pro anti-PD-1 ScFv. These EVs were incubated with activated CD4+ T cells for 36 hours, the cells stained for PD-1, and then analyzed by flow cytometry. Only XStamp Pro anti-PD-1 EVs are internalized by PD-1-expressing cells (compare bottom right panel to bottom left panel, blue arrow). (B) A comparison of the absolute cell count from each condition further demonstrates the specificity of the XStamp Pro anti-PD-1 EVs as the mistargeted EVs (decorated with XStamp Pro anti-HIV ScFv) were not taken up by the activated cells. *Data courtesy of Drs. Pooja Bhardwaj and Satish Pillai, UCSF*.

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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Ordering Information: orders@systembio.com

Licensing and Warranty Statement

Limited Use License

Use of XStamp Pro EV Targeting kits (*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.

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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.

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