

## Description

The VLP Builder Kit provides a streamlined solution for generating safe, biologically inert virus-like particles (VLPs). This system is optimized for expressing and displaying complex mammalian cell surface proteins—such as Tumor-Associated Antigens (TAAs) and G-protein coupled receptors (GPCRs)—in a convenient, water-soluble format. By leveraging the behavior of the lentiviral Gag protein, the kit facilitates the budding of particles from the lipid raft microdomains of Freestyle™ 293 cells. As these particles are secreted, they capture regions of the host plasma membrane, ensuring that membrane proteins are presented in their authentic cellular context and native conformation. This kit contains materials sufficient to purify five preparations of 50 ml of mammalian cell culture at a cell density of  $1 \times 10^6$  cells/ml.

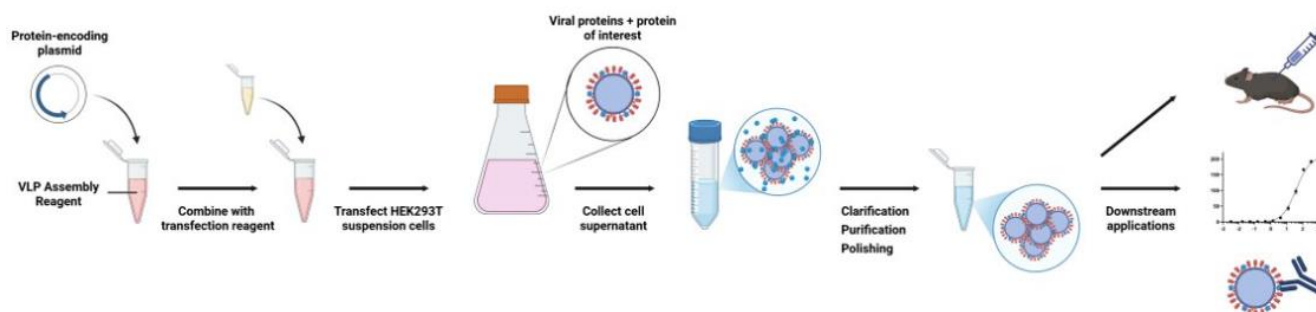


Figure 1. VLP expression and purification schematic.

## Background

Virus-like Particles (VLPs) are non-infectious, genome-free structures that provide a safer and more efficient alternative to traditional protein isolation methods. By expressing the HIV-1 Gag protein in cell lines including Freestyle™ 293 cells, this system leverages Gag's natural affinity for lipid raft microdomains—specialized membrane regions that regulate protein conformation and quality control. As these particles bud and secrete from the host cells, they selectively capture and envelop segments of the plasma membrane rich in native and recombinant proteins. This process preserves the target proteins in their authentic cellular context and orientation. Because VLPs are naturally released into the culture medium, researchers can harvest highly concentrated, receptor-rich preparations directly from the supernatant. This streamlined workflow eliminates the need for complex cell fractionation and detergent-based extraction, significantly reducing contaminants from internal organelles like the ER or Golgi.

## Applications

- Study membrane-associated proteins.
- Screen inhibitors and antibodies against membrane-associated proteins.

*\*There are many factors that affect protein yield, solubility, and function. Therefore, your expressed membrane protein might not be suitable for all the downstream applications listed above.*

## Supplied Materials

Catalog #	Name	Amount	Storage
83768-KC250 <sup>+</sup>	VLP Builder Reagent*	250 $\mu$ l	-20°C
83769-KC50 <sup>+</sup>	VLP Builder Precipitation Mix	50 ml	Room Temp
83770-KC750 <sup>+</sup>	VLP Transfection Reagent	750 $\mu$ l	4°C

*\*To avoid freeze-thaw cycles of the VLP Builder Reagent, it is recommended to make aliquots before storing them at -20°C.*

**Materials Required but Not Supplied**

- FreeStyle™ 293-F Cells (Thermo Fisher Scientific #R79007)\*
- FreeStyle™ 293 Expression Medium (Thermo Fisher Scientific #12338026)
- Opti-MEM™ I Reduced Serum Medium (Thermo Fisher Scientific #31985070)
- Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific #23225)
- PBS (Phosphate Buffered Saline)
- 37°C shaker with a humidified atmosphere of 8% CO<sub>2</sub>
- Refrigerated centrifuge with a swing rotor
- 250 ml Erlenmeyer flasks with vented caps
- Plasmid containing target protein under the EF1alpha promoter\*\*
- PBS containing 10% trehalose

\* This kit can also be used with similar cell lines.

\*\*We have seen a better effect using EF1alpha promoter than CMV promoter.

**Storage Conditions**

This kit will perform optimally for up to **6 months** from the date of receipt when the materials are stored as directed.

**Safety**

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

**VLP Expression and Purification Protocol**

- This protocol serves as a general guideline only.
- It is designed for the expression and purification of VLP proteins from a 50 ml cell culture.
- We recommend using a vector-only control for comparison.

**Day 1: Cells preparation**

1. Seed FreeStyle™ 293 cells at a density of 0.75 million cells/ml in a total volume of 50 ml using disposable 250 ml Erlenmeyer flasks with vented caps.
2. Incubate the cells overnight at 37°C in a shaking incubator with a humidified atmosphere of 8% CO<sub>2</sub>.

**Day 2: Transfection**

1. Count cells (they should be at 1.0-1.1 million cells/ml) and verify that cell viability is ≥98%.
2. In a sterile 15 ml tube, combine 50 µg of purified plasmid encoding the target protein, 50 µl of VLP Builder Reagent, and 2.5 ml of Opti-MEM™ I Reduced Serum Medium. Mix gently.
3. In a separate sterile 15 ml tube, combine 150 µl of VLP Transfection Reagent and 2.5 ml of Opti-MEM™ I Reduced Serum Medium. Mix gently.

4. Combine the diluted DNA/VLP Builder Reagent (from Step 2) with the diluted transfection reagent (from Step 3) and mix gently.
5. Incubate the mixture for 30 minutes at room temperature to allow complex formation.
6. Add the complexes dropwise to the 50 ml Freestyle™ 293 cell culture.
7. Incubate the cells for 96 hours at 37°C in a shaking incubator with a humidified atmosphere of 8% CO<sub>2</sub>.

**Day 6: Cells collection**

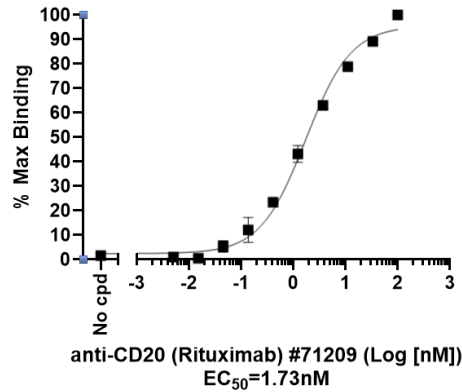
1. Transfer the entire culture to a 50 ml sterile tube and spin down at 3,000-5,000  $\times g$  at 4°C for 15 minutes.
2. Carefully transfer around 42 ml of supernatant using pipetting to a new 50 ml sterile tube and centrifuge at 3,000-5,000  $\times g$  for 15 minutes at 4°C.
3. Carefully transfer 40 ml of supernatant using pipetting to a new 50 ml sterile tube and add 9.6 ml of VLP Builder Precipitation Reagent.
4. Gently invert the tube 5–10 times to ensure homogeneous mixing.
5. Incubate the mixture overnight at 4°C without agitation.

**Day 7: VLP protein precipitation and storage**

1. Centrifuge the mixture at 3,000-5,000  $\times g$  for 15 minutes at 4°C.
2. Carefully remove the supernatant using pipetting.
3. Resuspend the pellets in 300-500  $\mu$ l of PBS containing 10% trehalose (or another suitable buffer).
4. Determine VLP protein concentration using Pierce™ BCA Protein Assay Kit.
5. Aliquot the resuspended VLPs into 5-10 vials to avoid freeze-thaw cycles and store at -80°C.

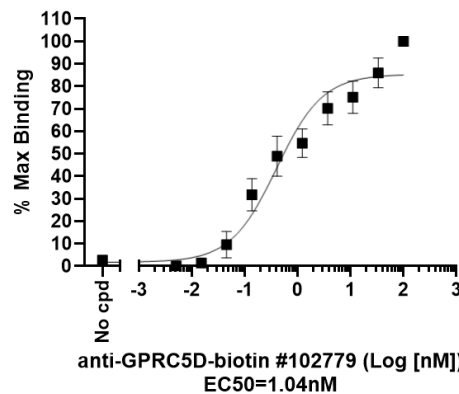
*Notes: For higher purity, further purification using ion-exchange chromatography or size-exclusion chromatography is recommended.*

*50 ml culture yields approximately 300  $\mu$ l of VLPs following resuspension. VLP protein concentration depends on protein expression and purification efficiency and may be between 1  $\mu$ g/ $\mu$ l and 2.5  $\mu$ g/ $\mu$ l.*

**CD20-VLP:anti-CD20 (Rituximab) Binding Assay**

*Figure 2. Binding between CD20-VLP protein and Anti-CD20 Functional Antibody (Rituximab).*

A 96 well plate was coated using 50  $\mu$ l of CD20-VLP at 10  $\mu$ g/ml per well overnight at 4°C. The plate was washed 3 times with 3x Immuno Buffer 1 (#79311) and blocked using 100  $\mu$ l of Blocking Buffer 2 (#79728) for 1 hour at room temperature. After removing the blocking buffer, 50  $\mu$ l/well of Anti-CD20 Functional Antibody (Rituximab) (#71209), serially diluted in Blocking Buffer 2, was added for 30 minutes at room temperature. After 3 more washes, the plate was incubated with Streptavidin-HRP, washed, and incubated with the Colorimetric HRP substrate. The reaction was stopped, and absorbance was read at 450 nm. The Blank value was subtracted from all values.

**GPRC5D-VLP:anti-GPRC5D-Bio Binding Assay**

*Figure 3. Binding between GPRC5D-VLP protein and biotinylated anti-GPRC5D antibody.*

A 96 well plate was coated using 50  $\mu$ l of GPRD5D-VLP at 20  $\mu$ g/ml per well overnight at 4°C. The plate was washed 3 times with 3x Immuno Buffer 1 (#79311) and blocked using 100  $\mu$ l of Blocking Buffer 2 (#79728) for 1 hour at room temperature. After removing the blocking buffer, 50  $\mu$ l/well of anti-GPRC5D antibody (#102779), serially diluted in Blocking Buffer 2, was added for 30 minutes at room temperature. After 3 more washes, the plate was incubated with Streptavidin-HRP, washed, and incubated with the Colorimetric HRP substrate. The reaction was stopped, and absorbance was read at 450 nm. The Blank value was subtracted from all values.

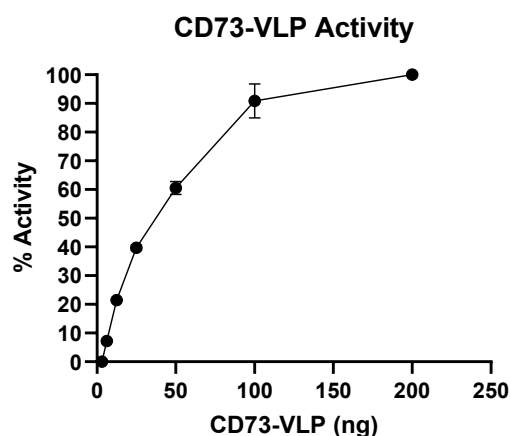


Figure 4. CD73-VLP activity.

CD73 is a cell surface protein with nucleotidase activity, which degrades AMP into adenosine. To assess the functionality of the enzyme-VLP, an enzymatic assay was performed according to CD73 Inhibitor Screening Assay Kit (#72055) with various amounts of CD73-VLP protein, demonstrating intact enzymatic activity for this VLP protein.

Data shown is representative.

### Troubleshooting Guide

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

### Related Products

Products	Catalog #	Size
Envelope VLP Control	102757	20 µg/ 100 µg
GPRC5D, VLP	102618	20 µg/ 50 µg
CD20, VLP	102671	5 µg/ 10 µg/ 50 µg

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