

GPCR VLPs & Nanodiscs for Drug Discovery

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Introduction

The G protein-coupled receptors (GPCRs) protein family, also known as seven-transmembrane receptors, represents a major class of drug targets. However, extraction and purification of membrane proteins like GPCRs, are generally challenging due to low expression levels and the hydrophobic nature of transmembrane segments. To tackle this challenge, we have engineered and produced GPCRs in Virus-like Particle (VLP) and Copolymer Nanodisc formats, which have been successfully applied to GPCR antibody drug discovery and various *in vitro* assays.

Full-length GPCRs displayed on VLPs

Virus-like particles (VLPs) are non-infectious particles that mimic the structure of viruses but do not contain genetic material. They are often as a tool of presenting multi-transmembrane proteins for various research purposes. KACTUS has successfully displayed specific GPCRs in a full-length, native conformation useful for stimulating immune

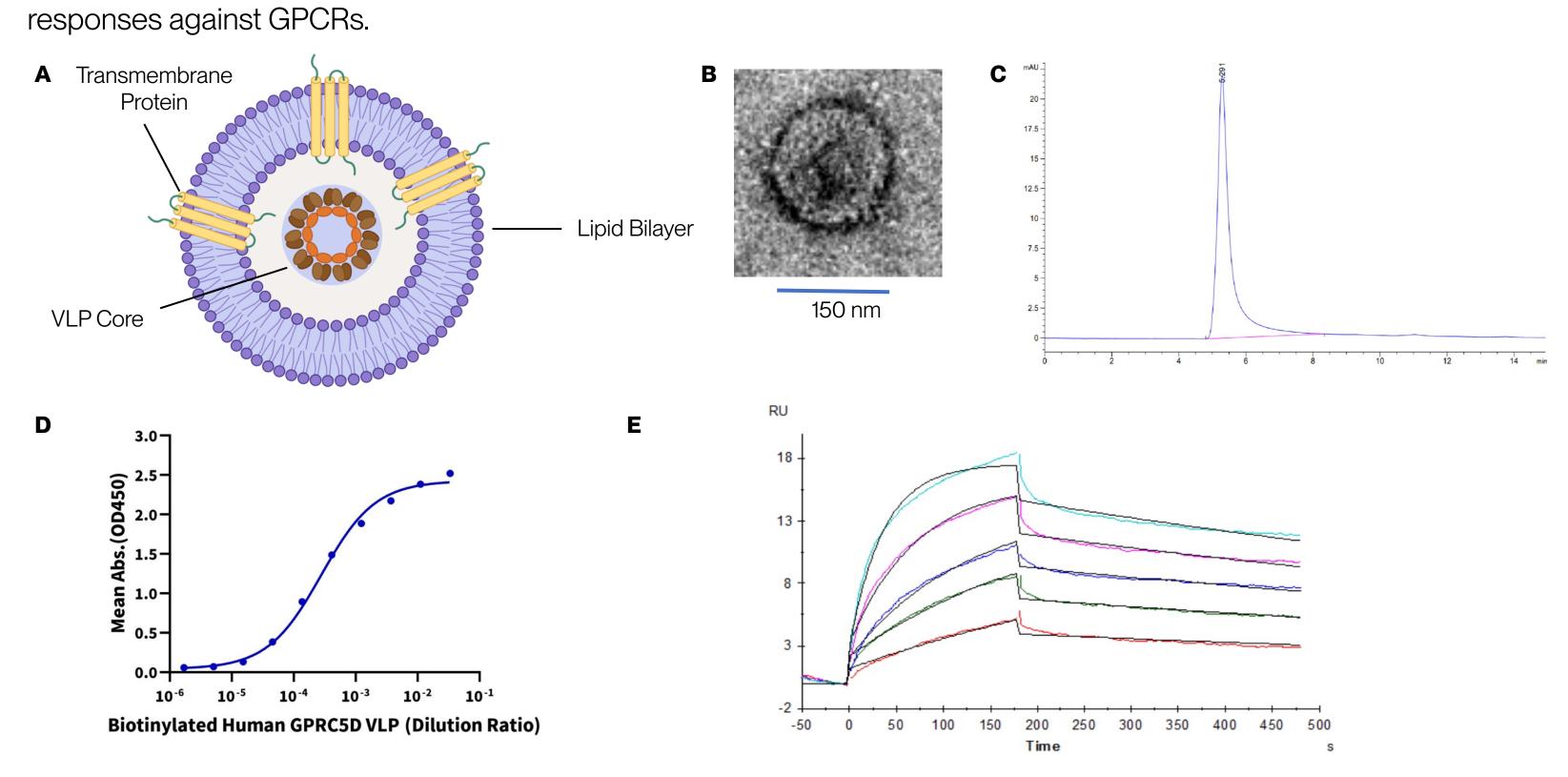


Figure 1. (A) Schematic diagram of GPCR displayed on an enveloped VLP. (B) GPRC5D VLPs are approximately 150nm diameter imaged using transmission electron microscope (TEM). (C) The purity of Human GPRC5D VLP is greater than 95% as determined by SEC-HPLC. (D) Immobilized Anti-GPRC5D Antibody (2μg/mL with 100μL/well) can bind various dilutions of Biotinylated Human GPRC5D VLP. (E) Biotinylated Human GPRC5D VLP captured on SA Chip can bind Anti-GPRC5D antibody, Tag with an affinity constant of 0.30 nM in SPR assay.

Full-length GPCRs assembled into Copolymer Nanodiscs

Nanodiscs have emerged as a powerful tool in functional and structural studies for membrane proteins. KACTUS GPCR nanodiscs are produced in a mammalian-cell based, detergent-free process. The GPCR transmembrane segments are stabilized in the center of the phospholipid bilayer surrounded by the copolymer, with intracellular and extracellular domain exposed. The assembled GPCR nanodiscs are soluble in aqueous media in a native-like bilayer environment that maintains the physiological function of GPCRs.

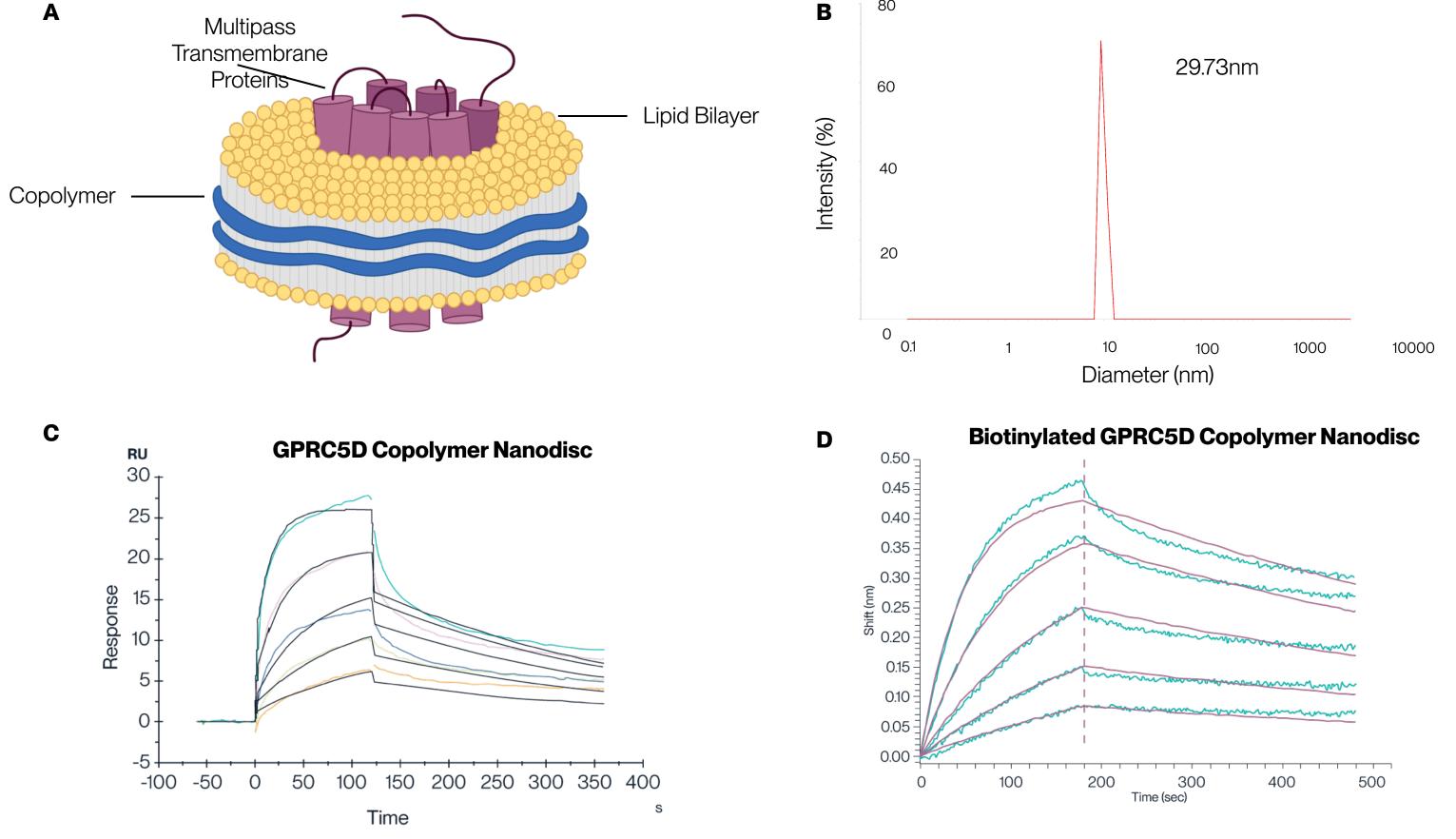


Figure 2. (A) Schematic diagram of GPCR assembled into a copolymer nanodisc. (B) The average size of Nanodiscs measured by DLS. (C) SPR analysis of Human GPRC5D Nanodiscs binding against anti-GPRC5D mAb ($K_D \sim 1.47$ nM) (D) High binding affinity of Biotinylated Human GPRC5D Nanodisc to anti-GPRC5D mAb ($K_D = 1.16$ nM), measured by BLI (Gator).

GPCR VLP & Nanodisc are suitable for phage panning

We tested our VLP and Nanodisc displayed GPCRs in ELISA to evaluate their potential for high-affinity interactions. Using human CCR8, we found both our VLP and Nanodisc produced robust ELISA signals, demonstrating their ability to support phage panning, a method that requires strong binding interactions for effective target enrichment.

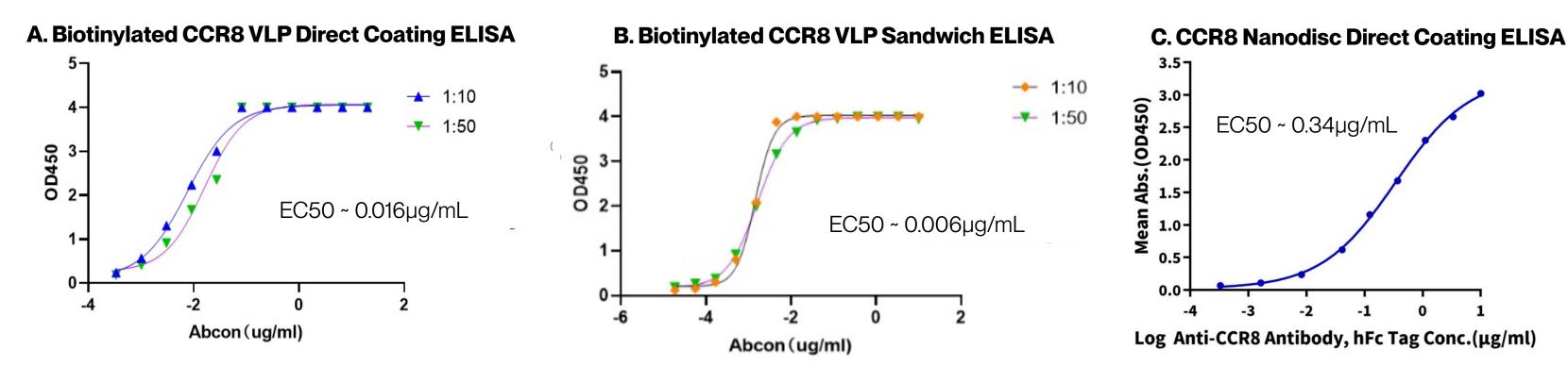


Figure 3. ELISA binding assays between CCR8 VLP (A), nanodisc and Anti-CCR8 monoclonal antibody Binding affinity of CCR8 VLP (0.4mg/mL, with 1:10 and 1:50 dilution, respectively) against Anti-CCR8 drug antibody. (B) Biotinylated CCR8 VLP (0.4mg/mL) was diluted 1:10 and 1:50 Anti-CCR8 drug antibody (10μg/mL) was added at a 3x serial dilution. (C) Immobilized Human CCR8 nanodisc (5μg/mL with 100μL/well) can bind Anti-CCR8 Antibody with an EC₅₀ of 0.34μg/mL in ELISA.

References

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GPCR VLP immunogens yielded functional antibodies with high affinity

KACTUS initiated an in-house immunization campaign using a CXCR4 Virus-like Particle (VLP) to evaluate its effectiveness in generating high-quality antibodies. CXCR4 is a G protein-coupled receptor (GPCR) that affects leukocyte migration and is overexpressed in over 23 types of cancer, making it a prominent target for antibody development in oncology research. The goal of this study was to determine the effectiveness of CXCR4 VLP for robust antiserum generation and to functionally validate the lead antibodies through ligand blocking assays.

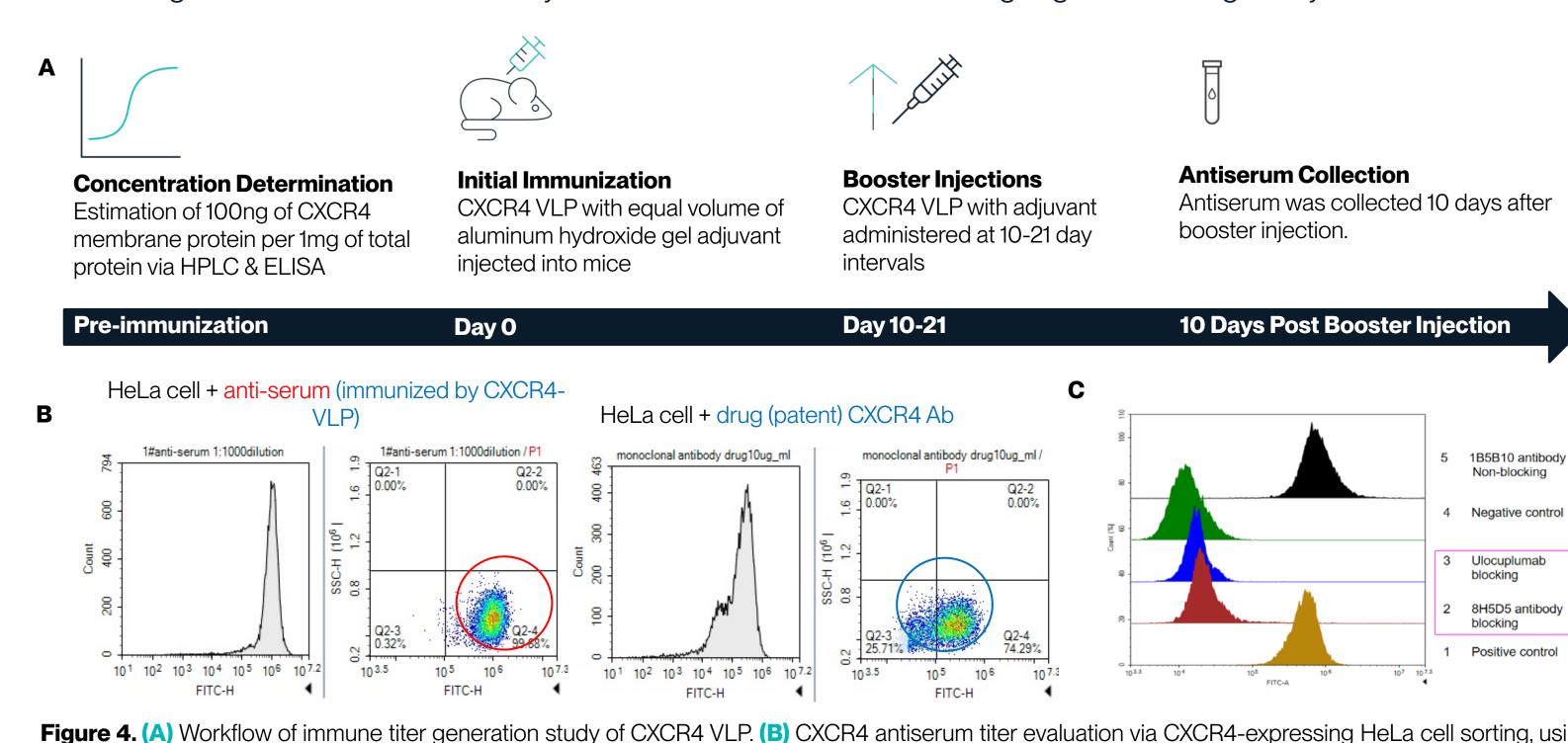
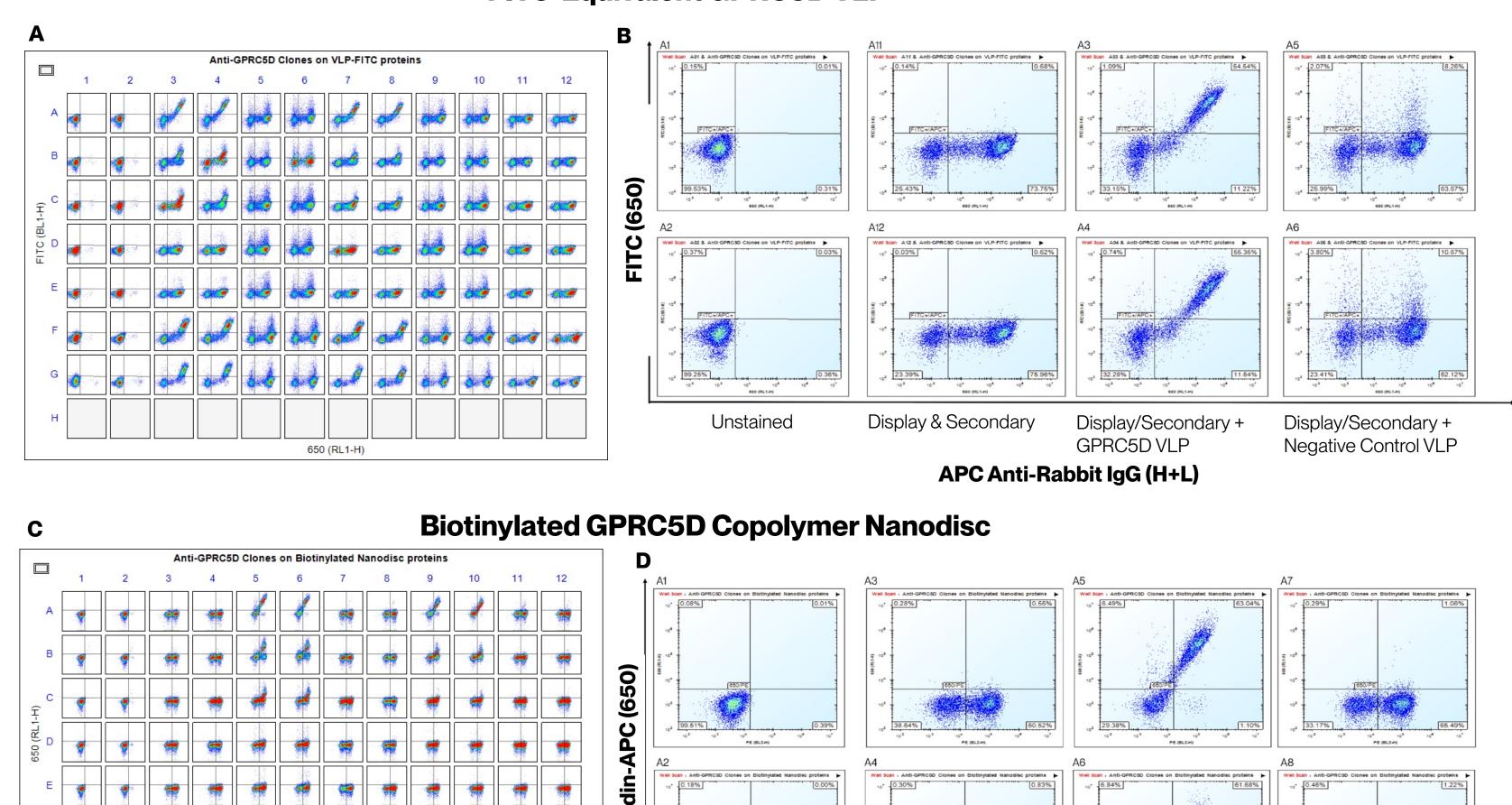


Figure 4. (A) Workflow of immune titer generation study of CXCR4 VLP. **(B)** CXCR4 antiserum titer evaluation via CXCR4-expressing HeLa cell sorting, using Ulocuplumab as positive control. **(C)** Flow cytometry analysis of ligand blocking by commercial and anti CXCR4 monolonal antibodies isolated in-house. The positive control (Panel 1) shows successful ligand binding to CXCR4. The 8H5D5 antibody (Panel 2) and the commercial blocking antibody Ulocuplumab (Panel 3) effectively blocked ligand binding, demonstrating a shift in fluorescence intensity similar to each other. The 1B5B10 antibody (Panel 5), classified as non-blocking, shows no inhibition of ligand binding, comparable to the negative control (Panel 4). This confirms that the 8H5D5 monoclonal antibody has similar blocking capability to the commercial CXCR4 blocking antibody.

GPCR VLPs & Nanodiscs are effective screening targets for antibody display on yeast

A leading antibody discovery company applied our GPRC5D VLP and nanodisc to yeast display technology to evaluate the binding efficiency and specificity of anti-GPRC5D antibodies. Yeast cells were engineered to display various antigen-binding fragments (Fab) and single-chain fragment variables (scFv). The full-length GPRC5D membrane protein was provided in two different formats: FITC-equivalent VLP and Biotinylated Copolymer Nanodisc. Both formats showed robust and specific selection against positive clones.

FITC-Equivalent GPRC5D VLP



PE Anti-Rabbit IgG (H+L)

Display/Secondary +

GPRC5D Nanodisc

Display/Secondary +

Negative Control Nanodisc

Display & Secondary

Figure 5. (A) Plate view for FITC-equivalent GPRC5D VLP binding to various mAb displayed on yeast. (B) FITC-equivalent GPRC5D VLP binds Rabbit Anti-GPRC5D Clone 1 Fab specifically, with significant dual-positive signals for both FITC and APC channels. (C) FACS plate view screening using Biotinylated Copolymer GPRC5D Nanodisc. (D) Biotinylated GPRC5D Nanodisc binds to Rabbit Anti-GPRC5D Clone 1 specifically Fab, with dual-positive signals (SA-APC and PE) observed.

Unstained

GPCRs Produced by KACTUS

PE (BL2-H)

We have successfully produced or in developing the following GPCRs. To request a sample or learn more, please contact us at support@kactusbio.us.

A2AR	CCR4	CNR1	CXCR5	GLP-1R	LGR4	PAR2
APLNR	CCR5	CNR2	CXCR6	GPR56	LGR5	S1PR5
BILF1	CCR6	CRTH2/PTGDR2	CXCR7	GPR75	LGR6	SMO
C5AR1	CCR7	CX3CR1	DRD1	GPR81	LPAR1	SSTR2
C5AR2/GPR77	CCR8	CXCR1	EDNRA	GPR84	MRGPRX2	SSTR4
CB1	CCR9	CXCR2	EDNRB	GPR87	NPSR1/GPR154	Steap-1
CB2	CCR10	CXCR3	GCGR	GPRC5D	OPRM1	-
CCR2b	CGRPR/RAMP1	CXCR4	GIPR	LGR3	PAR1	

Conclusion

- KACTUS proprietary membrane proteins in VLP and/or copolymer nanodisc formats are powerful tools for antibody discovery research and bioanalytical assays development and validation.
- Our GPCR VLPs with crucial post-translational modifications are effective immunogens for antibody discovery campaigns, as shown in our CXCR4 VLP immunization.
- Our GPCR VLPs and nanodiscs have been validated applicable for yeast panning, with solid potential for phage panning as well.

Acknowledgement

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