Application of GPRC5D VLP and Copolymer Nanodisc in Isolating GPRC5D Binders for Yeast Display Antibody Discovery

Background

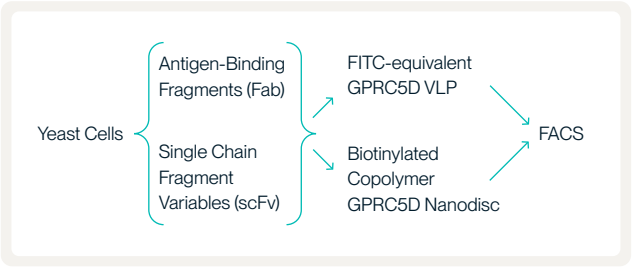
GPRC5D, a member of the G protein-coupled receptor (GPCR) family, has emerged as a promising target for therapeutic antibody development due to its selective expression in certain cancers, particularly multiple myeloma. However, targeting GPCRs like GPRC5D poses significant challenges, primarily due to their complex structure and membrane-bound nature. These proteins often exhibit conformational flexibility and are embedded within the cell membrane, making it difficult to preserve their native structure for antibody binding studies. Additionally, the presence of multiple transmembrane domains complicates the generation of antibodies with high affinity and specificity. Therefore, it is crucial to have full-length, functional GPRC5D protein with high structural integrity for effective therapeutic antibody development against GPRC5D.

At KACTUS, we have developed innovative strategies to address these challenges of targeting GPRC5D and other membrane proteins by leveraging Virus-Like Particles (VLPs) and copolymer Nanodiscs as powerful tools to display full-length proteins in native conformations. VLPs are non-infectious particles that mimic the structure of viruses, allowing us to present full-length proteins in a membrane-bound format. Similarly, our copolymer Nanodiscs use natural lipid bilayers and detergent-free extraction to display multi-transmembrane proteins. Both our VLP and Nanodisc membrane protein display platforms ensure that target antigens retain their native structure, allowing for more effective antibody discovery and screening.

Experiment Overview

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 (Cat No. GPR-HM0P11) and Biotinylated Copolymer Nanodisc (Cat No. GPR-HM45PB). The binding efficiency of the yeast-displayed anti-GPRC5D antibodies to the full-length GPRC5D membrane proteins presented in both VLP and Nanodisc formats was assessed using flow cytometry. Negative controls included both non-specific Fab fragments and Nanodiscs or VLPs without GPRC5D. Unstained samples were used as a baseline for autofluorescence.

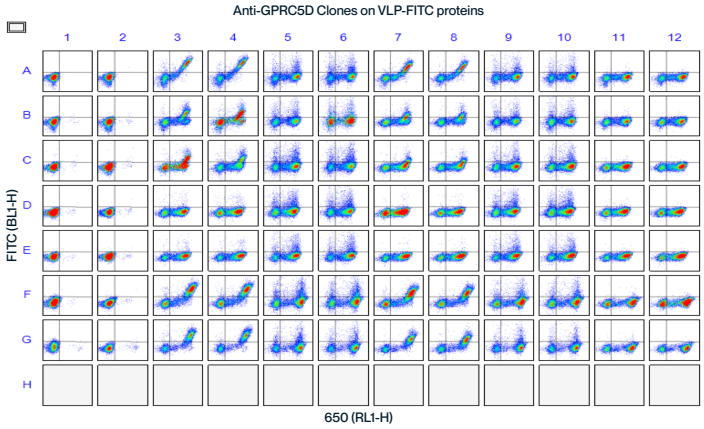
Products Used	
Product Description	**Catalog No.**
FITC-equivalent GPRC5D VLP	GPR-HM0P11
Biotinylated GPRC5D Copolymer Nanodisc	GPR-HM45PB



Summary of Results

Our VLP and Nanodisc effectively facilitated the identification of high-binding anti-GPRC5D antibody clones via yeast display. The results demonstrate that both the full-length GPRC5D VLP (Cat No. GPR-HM0P11) and GPRC5D Nanodisc (Cat No. GPR-HM45PB) showed strong and specific binding interactions with various monoclonal antibodies (mAbs) displayed on yeast cells, as evidenced by significant fluorescence signals across multiple antibody clones at different GPRC5D membrane protein concentrations. Specifically, the Rabbit Anti-GPRC5D Clone 1 Fab exhibited robust binding to both GPRC5D VLPs and Nanodiscs, with clear shifts in cell populations indicating dual-positive signals in the APC and FITC (for VLPs), PE and APC (for Nanodiscs) channels. Importantly, the GPRC5D Nanodisc displayed no binding to control yeast cells, underscoring its suitability for selective yeast display screening. **These results highlight the effectiveness of our VLP and Nanodisc products in screening for high-affinity antibodies against GPRC5D, providing customers with reliable tools for antibody discovery and validation.**

VLP Binding



Row	mAb-displayed on yeast
A	Rabbit Anti-GPRC5D Clone 1 Fab
B	Rabbit Anti-GPRC5D Clone 2 Fab
C	Rabbit Anti-GPRC5D Clone 3 Fab
D	Rabbit Anti-Negative Control 1 Fab
E	Rabbit Anti-Negative Control 2 Fab
F	Human Anti-GPRC5D Clone 4 scFv
G	Human Anti-GPRC5D Clone 5 scFv

Column	mAb-displayed on yeast
1-2	Unstained
3-4	100 nM GPRC5D VLP-FITC
5-6	100 nM Negative Control VLP-FITC
7-8	25 nM GPRC5D VLP-FITC
9-10	25 nM Negative Control VLP-FITC
11-12	Display and Secondary Detection Only

FITC: FITC
650: APC Anti-IgG

Figure 1. *Plate view for FITC-equivalent GPRC5D VLP binding various mAb displayed on yeast. The VLP plate showed distinct mAb binding (rows A, B, C, F) with strong FITC signals for 100nM GPRC5D VLP (columns 3-4), and 25nM GPRC5D VLP (columns 7-8), indicating successful binding of the antibodies to the GPRC5D presented on VLPs. Negative controls (rows D, E) exhibited low or no binding, confirming the specificity of the antibody interactions.*

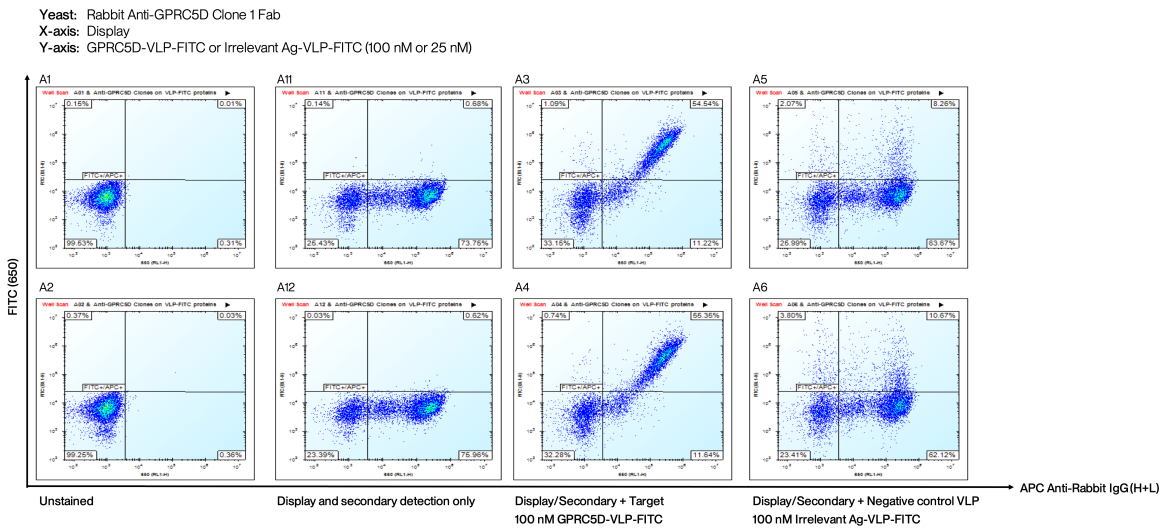
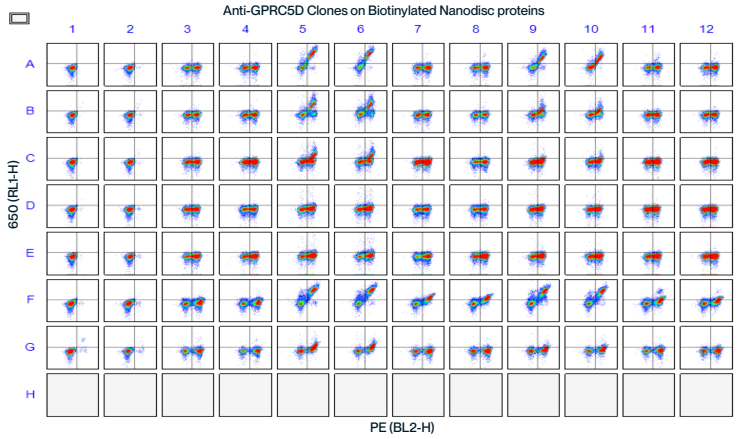


Figure 2. *FITC-equivalent GPRC5D VLP binding Rabbit Anti-GPRC5D Clone 1 Fab. The Rabbit Anti-GPRC5D Clone 1 Fab displayed on yeast cells binds FITC-equivalent GPRC5D VLP. In the unstained yeast cell (A1 and A2), no fluorescent signal was found in either the APC or FITC channel. Yeast cells stained with APC labeled anti-IgG antibody (A11 and A12) showed fluorescent signal in the APC channel, indicating yeast cells displaying Fab. When yeast displaying the Fab interacts with 100 nM GPRC5D-VLP-FITC (A3 and A4), there is a notable shift in the population to the upper right quadrant, demonstrating strong binding to the VLPs, with significant dual-positive signals for both FITC and APC channels. This indicates a successful and specific interaction between the Fab and GPRC5D on the VLPs. Conversely, when an irrelevant antigen VLP-FITC is used (A5 and A6), the binding is markedly reduced, with most cells remaining in the lower two quadrants, similar to the controls. This further confirms the specificity of the Rabbit Anti-GPRC5D Clone 1 Fab for GPRC5D presented on VLPs.*

Nanodisc Binding



Row	mAb-displayed on yeast
A	Rabbit Anti-GPRC5D Clone 1 Fab
B	Rabbit Anti-GPRC5D Clone 2 Fab
C	Rabbit Anti-GPRC5D Clone 3 Fab
D	Rabbit Anti-Negative Control 1 Fab
E	Rabbit Anti-Negative Control 2 Fab
F	Human Anti-GPRC5D Clone 4 scFv
G	Human Anti-GPRC5D Clone 5 scFv

Column	Antigen (in duplicates)
1-2	Unstained
3-4	Display and Secondary Detection Only
5-6	100 nM Biotinylated GPRC5D Nanodisc
7-8	100 nM Biotinylated Negative Control Nanodisc
9-10	25 nM Biotinylated GPRC5D Nanodisc
11-12	25 nM Biotinylated Negative Control Nanodisc

650: Streptavidin-APC
PE: PE Anti-IgG

Figure 3. *Plate view for Biotinylated GPRC5D Nanodisc binding various mAb displayed on yeast. Most mAb clones (rows A, B, C, F) showed significant binding to the Biotinylated GPRC5D Nanodiscs in the 100 nM (column 5-6) and 25 nM concentrations (column 9-10), as indicated by the fluorescence in both APC and PE channels. Negative controls (rows D, E) showed minimal or no binding, indicating specificity of the antibody clones for GPRC5D.*

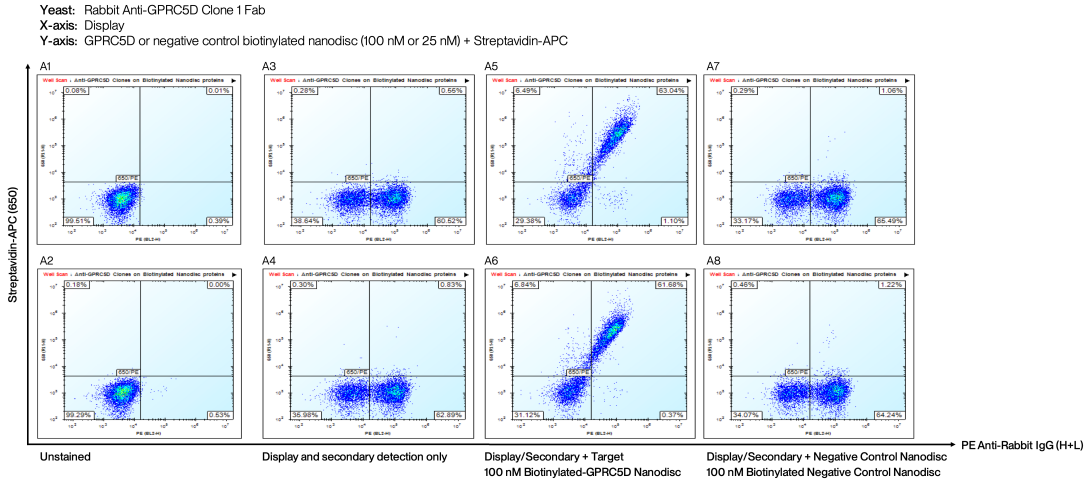


Figure 4. Biotinylated GPRC5D Nanodisc binding Rabbit Anti-GPRC5D Clone 1 Fab. *The Rabbit Anti-GPRC5D Clone 1 Fab displayed on yeast cells binds to Biotinylated GPRC5D Nanodisc. In the unstained yeast cells (A1 and A2), no fluorescent signal was found in either the APC or PE channel. Yeast cells stained with PE-labeled anti-IgG antibody (A3 and A4) showed fluorescent signal in the PE channel, indicating yeast cells displaying Fab. When yeast cells displaying the Fab interact with 100 nM biotinylated GPRC5D Nanodiscs (A5 and A6) and are stained with streptavidin-APC, signal is observed in the APC channel. This indicates a successful and specific interaction between the Fab and GPRC5D Nanodisc. In contrast, when negative control Nanodiscs are used (A7 and A8), and stained with streptavidin-APC, there is no signal in APC channel. These results confirm the high specificity of Rabbit Anti-GPRC5D Clone 1 Fab for the GPRC5D Nanodiscs.*

Conclusion:

KACTUS FITC-equivalent GPRC5D VLP (Cat No. GPR-HM0P11) and Biotinylated GPRC5D Nanodisc (Cat No. GPR-HM45PB) were successfully used to isolate GPRC5D binders using for yeast display in antibody discovery. Our VLP and Nanodisc membrane protein expression platforms provide a robust and versatile solution for presenting full-length membrane proteins in their native conformations. This enables high-specificity screening and selection of therapeutic antibody candidates, ultimately accelerating the antibody discovery process and enhancing the development of targeted therapies.