GPCR PAR1 or DRD2-Specific Monoclonal Antibodies Can Detect the Target Expressed on Live Cell Surfaces by Flow Cytometry

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Abstract

Antibody staining of G protein-coupled receptors (GPCRs) on live cells is important for studying receptor expression, localization, and dynamics in their native, functional state. This approach provides a more accurate view of receptor behavior than methods that use fixed cells, which can cause artifacts and alter the receptor's conformation. Due to their complex membrane environment, low native expression levels, and dynamic conformations, GPCRs can be difficult targets for antibodies. We have developed proprietary fit-for-target immunization approaches to generate GPCR-specific antibodies in mice. The immune mouse B cells are used for monoclonal antibody discovery for target staining on live cell surface by flow cytometry. Protease-activated receptor-1 (PAR1) and Dopamine receptor D2 (DRD2) are Class A GPCRs. PAR1- or DRD2-specific monoclonal antibodies (mAbs) were generated from immunized mice. The mAbs could stain PAR1 or DRD2 expressed on live cell surfaces by flow cytometry. As antibody staining of live GPCRs is a foundational and indispensable technique, these mAbs can be useful tools for PAR1 and DRD2 research and drug discovery.

Anti-PAR1 mAb (Conigen Bioscience, CABh-24076); Anti-DRD2 mAb (Conigen Bioscience, CABh-24075)

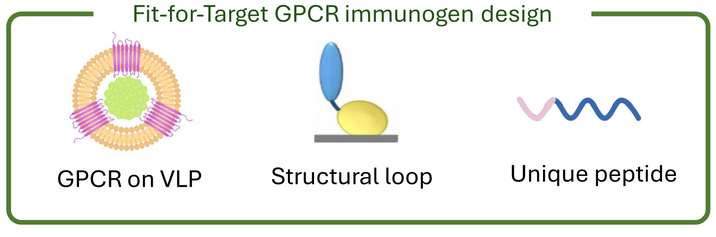


Fig 1. Conigen's proprietary rational immunogen and immunization regimen design to generate antibodies against GPCR.

Protease-activated receptor-1 (PAR1)

PAR1-specific antibodies elicited by immunization in mice

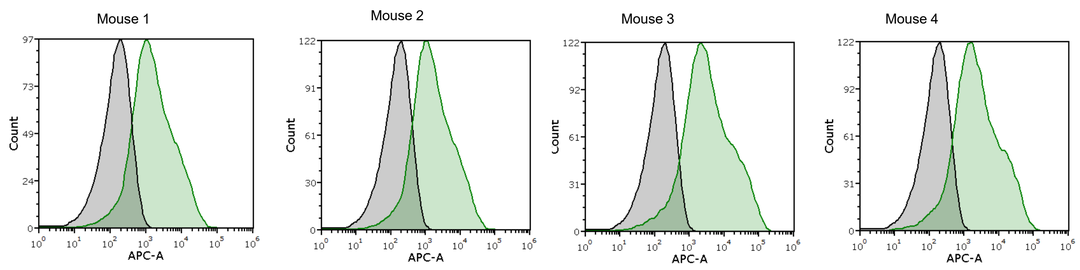


Fig 2. PAR1-specific antibodies in individual immune mouse sera binding to PAR1 expressing HEK293 cells by Flow Cytometry. Green curve: immune mouse sera; grey curve: pre-immune sera.

PAR1-specific monoclonal antibody

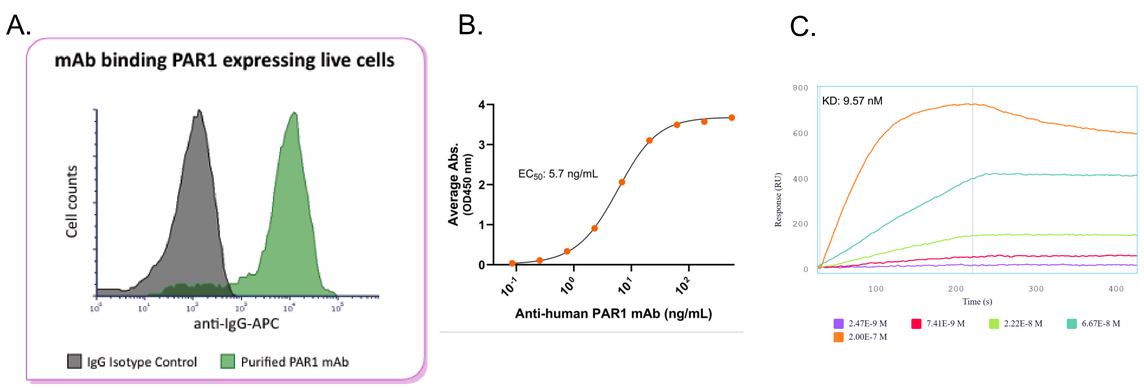


Fig 3. **PAR1-specific monoclonal antibody** potently binding PAR1 expressing HEK293 cells by Flow Cytometry (A), PAR1 target protein measured by ELISA (B) and SPR (C).

Dopamine receptor D2 (DRD2)

DRD2-specific antibodies elicited by immunization in mice

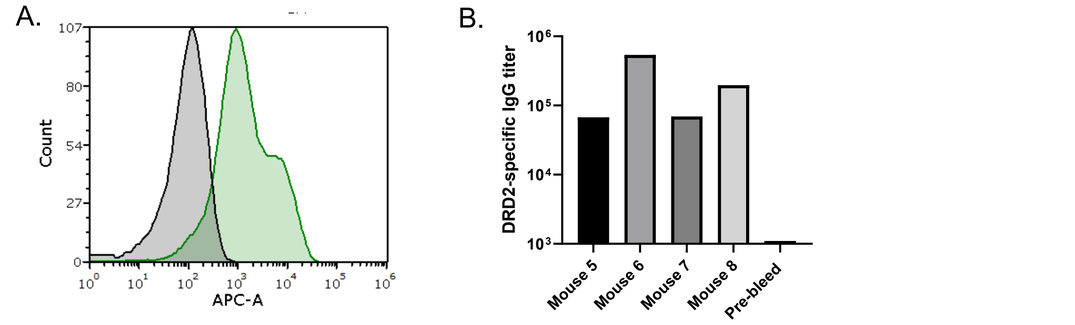


Fig 4. DRD2-specific antibody elicited in immune mouse serum. A. Immune mouse sera binding to DRD2 expressing HEK293 cells by flow cytometry. Green curve: immune mouse sera; grey curve: pre-immune sera. .B. Target-specific antibody titer measured by ELISA. .

DRD2-specific monoclonal antibody

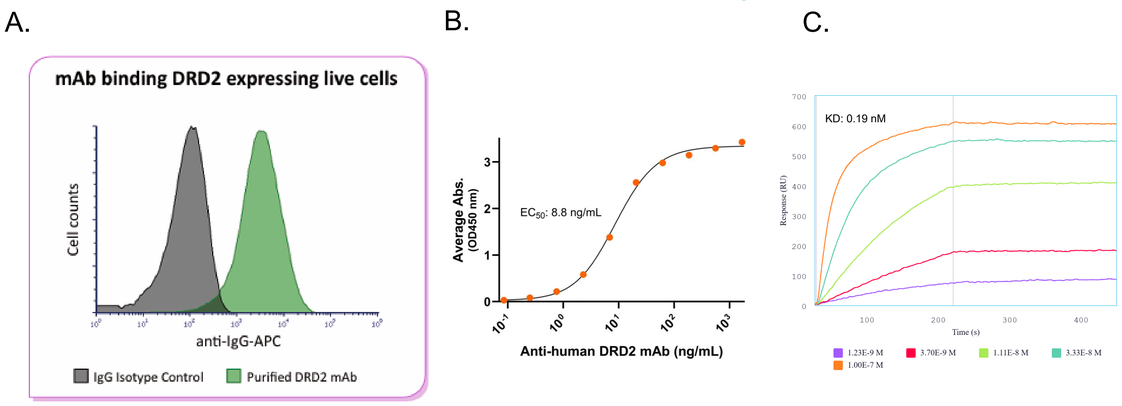
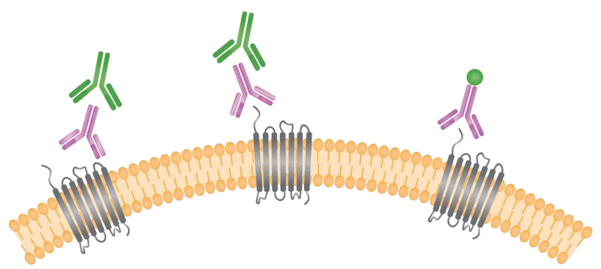


Fig 5. **DRD2-specific monoclonal antibody** potently binding DRD2 expressing HEK293 cells by Flow Cytometry (A), DRD2 target peptide measured by ELISA (B) and SPR (C).



Conclusions

- Rational GPCR immunogen design and proprietary immunization platforms can generate potent antibody responses in mice against GPCR protein target on live cell surface.
- **PAR1-specific monoclonal antibody** can specifically detect PAR1 target protein expression on live cells by flow cytometry.
- **DRD2-specific monoclonal antibody** can specifically detect DRD2 target protein expression on live cells by flow cytometry.
- The PAR1 and DRD2 specific mAbs can be useful tools for research and drug discovery.

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