

**MONOCLONAL ANTIBODY**

# Anti-GRK5 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
W077-3	2F11C3	Mouse IgG2a $\kappa$	100 $\mu$ L	1 mg/mL

**BACKGROUND:** G protein-coupled receptor kinase 5 (GRK5) is a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the Ser/Thr protein kinase family. GRK5 is expressed ubiquitously in human tissues including the heart, placenta, lung, skeletal muscle, brain, liver, pancreas, and kidney. GRK5 phosphorylates the activated forms of G protein-coupled receptors, facilitates receptor desensitization and internalization, and leads to receptor downregulation. High expression levels of GRK5 are observed in an aggressive prostate cancer PC3 cell line.

**SOURCE:** This antibody was purified from hybridoma culture supernatant by Protein A affinity column chromatography.

**IMMUNOGEN:** Human GRK5 expressed Ba/F3 transfectants generated from SST-REX (signal sequence trap by retrovirus-mediated expression screening).

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human GRK5 on Flow cytometry.

**APPLICATIONS:**

- Flow cytometry; 1-10  $\mu$ g/mL
- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested

Detailed procedure is provided in the following **PROTOCOL**.

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

**Entrez Gene ID:**

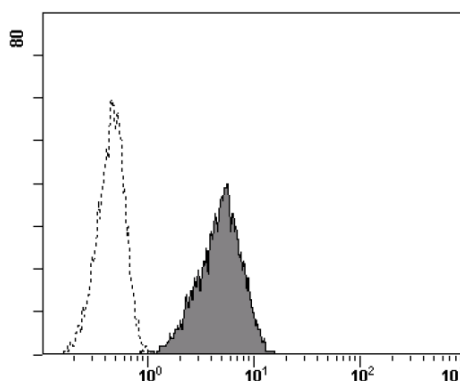
2869 (Human)

**REFERENCES:**

- 1) Baameur, F., *et al.*, *Mol. Pharmacol.* **77**, 405-415 (2010)
- 2) Fan, J. and Malik, A. B., *Nature Med.* **9**, 315-321 (2003)
- 3) Kojima, T. and Kitamura, T., *Nat. Biotechnol.* **17**, 487-490 (1999)
- 4) Kunapuli, P. and Benovic, J. L., *PNAS* **90**, 5588-5592 (1993)

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant	Not tested	Not tested	Not tested
Reactivity on FCM	+			



**Flow cytometric analysis of human GRK5 expression on Ba/F3 transfectant.** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of W077-3 to the cells.

**PROTOCOL:**

**Flow cytometric analysis for floating cells**

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.05%  $\text{NaN}_3$ ].
- 2) Resuspend the cells with washing buffer ( $2.5 \times 10^6$  cells/mL).
- 3) Add 200  $\mu$ L of cell suspension into each tube. And centrifuge at 500 x g for 1 minute at room temperature ( $20\sim 25^{\circ}\text{C}$ ). Remove supernatant by careful decantation.
- 4) Add 20  $\mu$ L of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after

tapping. Mix well and incubate for 5 minutes at room temperature.

- 5) Add 50  $\mu$ L of the primary antibody at the concentration as suggest in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful decantation.
- 7) Add 50  $\mu$ L of 1:200 anti-mouse IgG-PE (Beckman Coulter; code no. IM0855) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful decantation.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; transfectant)

## RELATED PRODUCTS:

CY-SPG03 GRK5 (Human), Active

W005-3	Anti-BTN2A1 (Human) mAb
W008-3	Anti-Carboxypeptidase D (Human) mAb
W010-3	Anti-CCDC107 (Human) mAb
W011-3	Anti-Dystroglycan (Human) mAb
W017-3	Anti-EphA2 (Human) mAb
W029-3	Anti-IGFBP1 (Human) mAb
W031-3	Anti-IGFBP6 (Human) mAb
W039-3	Anti-MANSC1 (Human) mAb
W041-3	Anti-Neuroplastin (Human) mAb
W046-3	Anti-CD201 (EPCR) (Human) mAb
W049-3	Anti-QSOX1 (Human) mAb
W050-3	Anti-RECK (Human) mAb
W052-3	Anti-Osteopontin (SPP1) (Human) mAb
W072-3	Anti-CD358 (DR6) (Human) mAb
W074-3	Anti-CRELD1 (Human) mAb
W077-3	Anti-GRK5 (Human) mAb
W080-3	Anti-ADAMTS1 (Human) mAb
W086-3	Anti-LYPD3 (C4.4A) (Human) mAb
W089-3	Anti-C11orf24 (Human) mAb
W109-3	Anti-TMED2 (Human) mAb
W111-3	Anti-DLL4 (Human) mAb
W117-3	Anti-TINAGL1 (Human) mAb
W124-3	Anti-GPR56 (Human) mAb
W125-3	Anti-GPR56 (Human) mAb
W128-3	Anti-CD318 (CDCP1) (Human) mAb
W147-3	Anti-TYRO3 (Human) mAb
W158-3	Anti-HEXA (Human) mAb
W164-3	Anti-RHBDD3 (Human) mAb
W172-3	Anti-CD172a (SIRP $\alpha$ ) (Human) mAb
W181-3	Anti-Apolipoprotein D (Human) mAb
W194-3	Anti-FAM171A1 (Human) mAb
W253-3	Anti-Glypican 1 (Human) mAb
W321-3	Anti-FGFRL1 (Human) mAb
W357-3	Anti-CD105 (Endoglin) (Human) mAb
W358-3	Anti-CD300A (Human) mAb
W359-3	Anti-CD300C (Human) mAb
M076-3	Mouse IgG2a (isotype control)
MTG-001	Clear Back (Human Fc receptor blocking reagent)