

Host Gqz5-Coupled CHO Cells (CHO/Gqz5)

Order Number: RD-HGQZ5(HYG)

Cell Density and Storage: 2.4×10^6 cells frozen in 80 μ l of DMSO-free freezing medium. Cells must be stored in liquid nitrogen if not immediately processed upon receipt.

Note: A License from Molecular Devices Corporation is required prior to purchasing or using the cell line.

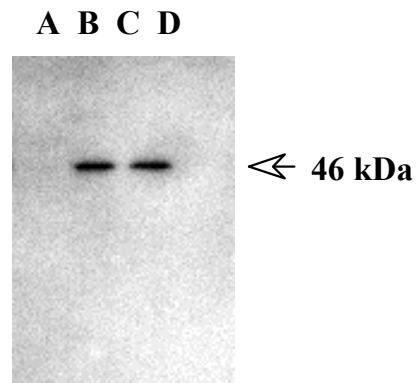
A. Description

CHO/Gqz5 is a CHO cell line stably expressing the chimeric Gqz5 alpha subunit protein. When used as a host cell for transfection expression of Gi/o-coupled receptors, the constitutively expressed Gqz5 protein in the cells allows many transfected receptors, which normally act through the cAMP pathway, to couple to Gq signal transduction and mobilize intracellular calcium. The cell line carries the hygromycin resistance gene and is resistant to hygromycin B.

A.1. Expression of Chimeric Gqz5 Alpha Subunit in CHO/Gqz5 Host Cells

CHO cells constitutively expressing the Gqz5 alpha subunit protein were produced by stable transfection of the cells with Gqz5 cDNA. The Gqz5 cDNA contains an internal HA epitope which allows detection of the expressed protein by anti-HA antibody. Expression of the 46 kDa chimeric Gqz5 alpha subunit protein in the cells was determined by Western blot analysis using a peroxidase-labeled anti-HA antibody.

Figure 1. Immunoblot detection of chimeric Gqz5 alpha in CHO/Gqz5 cells. Western blot analysis of cell extract was performed with an anti-HA-peroxidase mouse monoclonal antibody. The blot was developed using a chemiluminescent peroxidase detection kit. A & D = negative control, B & C = CHO/Gqz5.



A.2. Functional Coupling of Gi/o-coupled Receptors to Calcium Mobilization in CHO/Gqz5 Cells

Functional testing was carried out by transient transfection of Gz/o-coupled receptor cDNA into the CHO/Gqz5 host cells and measuring the stimulation of intracellular calcium mobilization by agonists. Figure 2 shows the result obtained when the Gi-coupled nociceptin receptor was transiently expressed in CHO/Gqi5 cells.

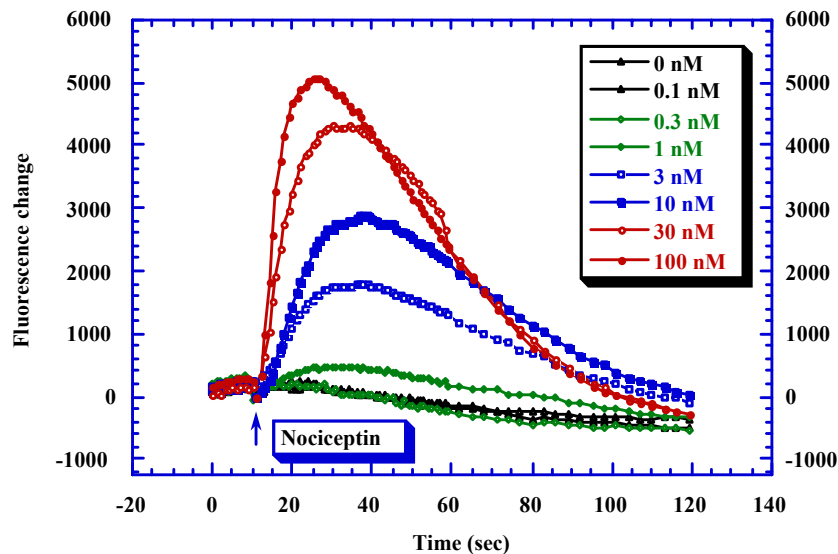


Figure 2. Coupling of nociceptin receptor to intracellular calcium mobilization in CHO/Gqz5 cells. Human nociceptin receptor cDNA was transiently transfected into CHO/Gqz5 using Lipofectamine. The transfected cells were loaded with Fluo-3 and nociceptin-mediated changes in intracellular calcium were measured by the FLIPR instrument (Molecular Devices Corporation, Sunnyvale, CA).

B. Thawing and Propagation of CHO/Gqz5 Cells

B.1. Culture Medium

Ham's F12 culture medium containing 10% fetal bovine serum, 100 unit/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 300 µg/ml hygromycin B.

B.2. Thawing and Seeding

1. Remove cell vial from the liquid nitrogen container.

2. Place vial in a 37°C heating block and immediately add 80 µl of culture medium to the vial . Allow to thaw, mix gently, and equilibrate at 37°C for 5 min.
3. Dilute the thawed cells with 0.5 ml of culture medium and seed into a T-75 flask containing 25 ml of culture medium. Grow the cells in a CO₂ incubator at 37°C.

B.3. Subculture and Propagation

1. Split the cells 1:10 every 3 to 4 days, using trypsin-EDTA to harvest the cells.
2. It is strongly recommended that a frozen cell bank be established soon after thawing the cells and establishing the initial cultures.