

## Host Gqo5-Coupled CHO Cell (CHO/Gqo5)

Order Number: RD-HGQO5

Cell Density and Storage: 2.4 x 10<sup>6</sup> cells frozen in 80 µl 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.**

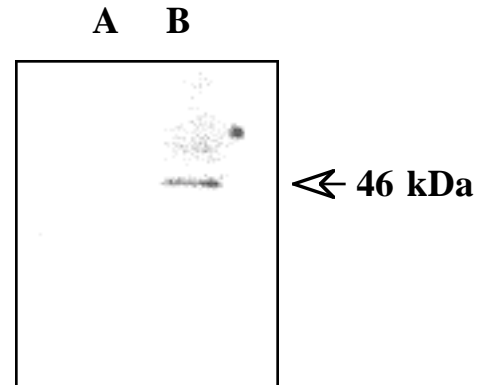
### A. Description

CHO/Gqo5 is a CHO cell line stably expressing the chimeric Gqo5 alpha subunit protein. When used as a host cell for transfection expression of Gi/o-coupled receptors, the constitutively expressed Gqo5 protein in the cells allows many transfected Gi/o-coupled 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 Gqo5 Alpha Subunit in CHO/Gqo5 Host Cells

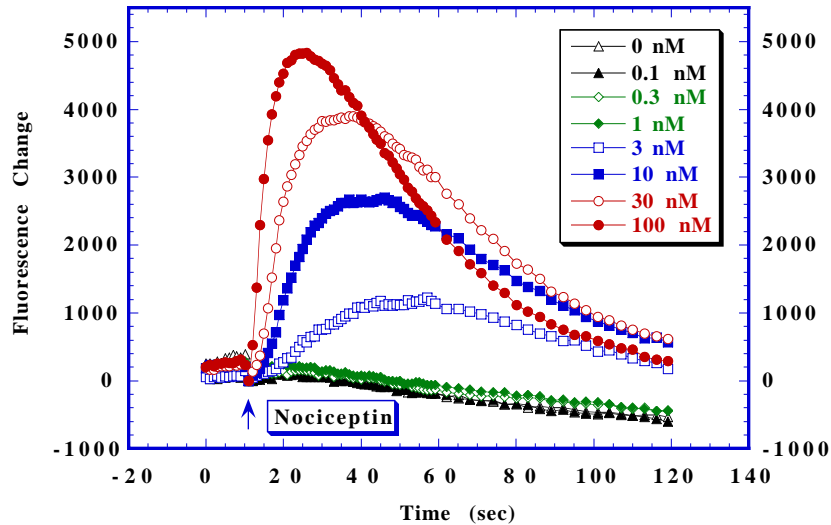
CHO cells constitutively expressing the Gqo5 alpha subunit protein were produced by stable transfection of the cells with Gqo5 cDNA. The Gqo5 cDNA contains an internal HA epitope which allows detection of the expressed protein by anti-HA antibody. Expression of the 46 kDa chimeric Gqo5 alpha subunit protein in the cells was determined by Western blot analysis using a peroxidase-labeled anti-HA antibody.

**Figure 1. Immunoblot detection of chimeric Gqo5 alpha in CHO/Gqo5 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 = negative control, B = CHO/Gqo5.



## A.2. Functional Coupling of Gi/o-coupled Receptors to Calcium Mobilization in CHO/Gqo5 Cells

Functional testing was carried out by transient transfection of Gi/o-coupled receptor cDNA into the CHO/Gqo5 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/Gqo5 cells.



**Figure 2. Coupling of nociceptin receptor to intracellular calcium mobilization in CHO/Gqo5 cells.** Human nociceptin receptor cDNA was transiently transfected into CHO/Gqo5 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/Gqo5 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 CO<sub>2</sub> 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.