Screen Quest™ CHO-$G_{\alpha 16}$ Chimera Cell line

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 38105 (1 Vial)	Keep in liquid Nitrogen	FLIPR, FDSS, FlexStation, NOVOStar, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

Screen Quest™ cell lines are a series of cells that have been successfully used in drug discovery and screening environments for studying G-protein-coupled receptors (GPCR) that do not conventionally couple through intracellular calcium. It has been effectively used with the FLIPR, FDSS Systems in conjunction with non-G_q coupled members of many receptors such as chemokine, serotonin, glutamate, dopamine, opioid, vasopressin and α- and ß- adrenergic receptor families. Over 60% of the known GPCR signal through pathways other than G_q which lead to an increase in intracellular calcium, and as genomics reveals more G-protein-coupled receptor targets this trend continues to increase. Screen Quest™ cell lines are used for investigating GPCR that do not conventionally couple through intracellular calcium.

Screen Quest™ cell lines are based on a series of G-protein chimeras, including the promiscuous G-protein, $G_{\alpha 16}$. The chimeras consist of the alpha subunit of a G_q-protein complex whose 5 carboxy-terminal amino acids have been replaced with those from one of the other G-proteins (either $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha o}$, or $G_{\alpha z}$). These amino acids are responsible for the coupling of the receptor to its G-protein. Co-expression of these chimeras with specific non-G_q-coupled receptors which normally act through the cAMP pathway may result in the generation of an intracellular calcium signal upon receptor stimulation.

Screen Quest™ CHO-$G_{\alpha 16}$ cell line is CHO-K1 cells stably transfected with the promiscuous G-protein, $G_{\alpha 16}$. When used as a host cell for transfection expression of Gi or Gs-coupled receptors, the constitutively expressed $G_{\alpha 16}$ protein in the cells allows the transfected receptor which normally act through the cAMP pathway, to couple to Gq signal transduction and mobilized intracellular calcium. Activation of the specific non-G_q-coupled receptors in these cells by specific ligands can be detected using calcium sensitive dyes such as Calbryte 520 AM, Cal-520 AM, Fluo-8 AM, or Fluo-4 AM and no wash calcium kits.

Handling Procedure for Frozen Cells

1. Cell Density and Storage

The cells are frozen at a density of 2 X 10^6 cells in 90% fetal bovine serum and 10% (v/v) DMSO. To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. Cells must be stored in liquid nitrogen if not immediately processed upon receipt.

2. Cell Culture Medium

Basal medium: Ham's F12 culture medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ mL penicillin, 100 µg/mL streptomycin (Basal medium).
Selection Medium: Basal Medium with 200 µg/mL hygromycin B.

3. Thawing and Seeding Cells

1) Prepare Basal Medium. Prepare 37 ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37 ºC water bath. Remove the vial from the water bath as soon as the contents are thawed, and sterilize the exterior of the vial with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
2) Add vial contents to 25 mL cell culture medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37 ºC, 5% CO_2.
3) All live cells should be attached after 18-24 hours post–thaw. Change Basal Medium with Selection Medium.

4. Subculture and Propagation

1) When cells are approximately 80% confluent, passage the cells.
2) Passage the cells 1:10 every 3-4 days, using Trypsin-EDTA to dissociate the cells.
3) It is highly recommended that a frozen cell bank be established at low passage number.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Data Analysis

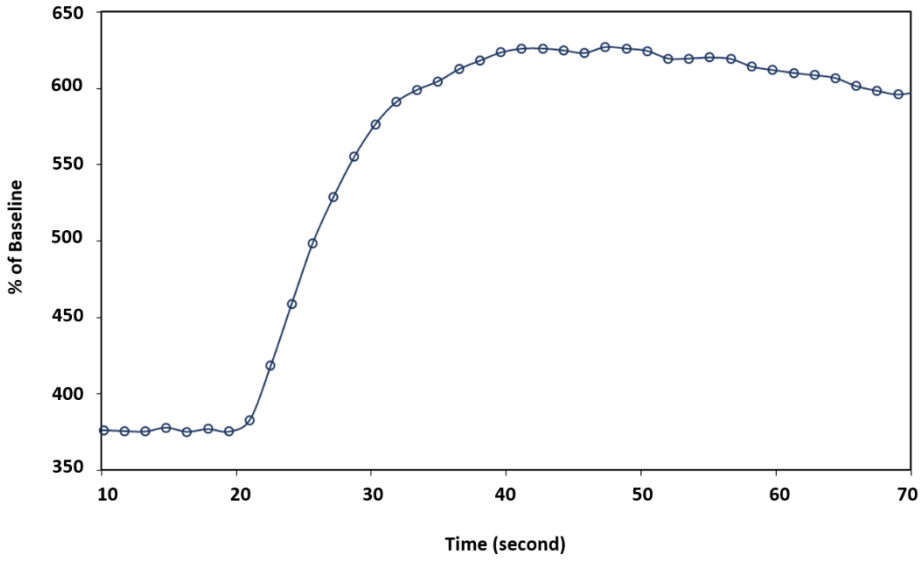

Figure 1. Nociceptin-stimulated calcium response was measured in CHO-Gα16 cells transiently transfected with Nociceptin receptor using Cal-520®, AM (Cat#21130)**.** Nociceptin receptor cDNA was transiently transfected into CHO-Gα16 cells. The transfected cells were incubated with equal volume (100 µL) of 10 µM Cal-520® AM with 2 mM probenecid in Hanks with 20 mM Hepes buffer (HHBS) at 37 °C for 1 hour. The Cal-520® AM loading solution were replaced with HHBS with 1 mM Probenecid. Nociceptin was added by FlexStation (Molecular Devices) to achieve the final concentration of 300 nM.