

Screen Quest™ CHO-Ga16-NOP Cell

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 38100 (1 Vial)	Keep in liquid Nitrogen	FLIPR, FDSS, FlexStation, NOVOSTar, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

Over 60% of the known G-protein-coupled receptors (GPCR) signal through pathways other than G_q which lead to an increase in intracellular calcium. As genomics reveals more G-protein-coupled receptor targets this trend continues to increase. Screen Quest™ cell lines enable to investigate 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_{a16} . 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_{as} , G_{ai} , G_{ao} , or G_{az}). 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 may result in the generation of an intracellular calcium signal upon receptor stimulation.

Screen Quest™ CHO-Ga16-NOP cell line is CHO-K1 cells stably transfected with both the promiscuous G-protein, G_{a16} and one of the three human opioid receptors, nociception receptor (also known as ORL-1). The constitutively expressed G_{a16} protein in the cells allows this receptor which normally act through the cAMP pathway, to couple to G_q signal transduction and mobilized intracellular calcium. Activation of the opioid/nociception 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 corresponding no wash calcium kits. This cell line has been successfully used in drug discovery and screening environments for studying GPCR that do not conventionally couple through intracellular calcium. It has been effectively used with the FLIPR, FDSS Systems.

Handling Procedure for Frozen Cells

1. Cell Density and Storage

The cells are frozen at a density of 2×10^6 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 and 400 µg/mL G418.

3. Thawing and Seeding Cells

- 1) Prepare Basal Medium. Prepare 37 °C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37 °C 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 °C, 5% CO₂.
- 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.

Data Analysis

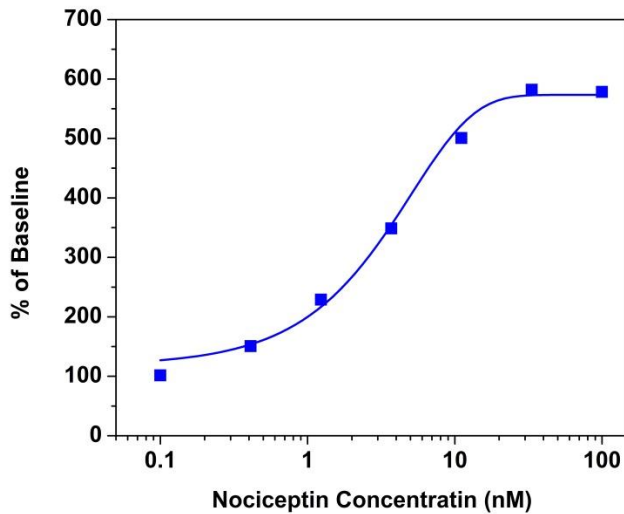


Figure 1. Nociceptin-stimulated calcium response was measured in CHO-G α 16-NOP cells with Cal-520®, AM (Cat#21130). CHO-G α 16-NOP cells were seeded overnight at 60,000 cells/100 μ L/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with equal volume (100 μ L) of 10 μ M 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 indicated concentrations.

Warning: This product shall be only sold to our authorized distributors and end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.