Innoprot
Speeding up drug discovery

ASSAY PROCEDURES

GLP-1R $_{cAMP}$Nomad HEK293 cell line

Catalog #: P70503

1. Introduction

Nomad cAMP assays are used to investigate cAMP cellular signaling pathway and as high-throughput screening tool for drug discovery. Nomad-cAMP HEK293 cell line is a clonal derivative of HEK293 cells. Glucagon Like Peptide 1 Receptor (GLP1R) cAMP Nomad cell line is designed for High throughput screening (HTS) analysis of the receptor response that results in a cellular cAMP signaling pathway regulation. The elevation of intracellular cAMP levels leads to a change in the cellular localization of the Biosensor and to an increment in the intensity of the signal.

2. Product Components and Storage Conditions

- **Product**: GLP1R $_{cAMP}$Nomad HEK293 cat.nº P70503

- **Size:** 2 vials $3x10^6$cells in Cryostor CS10 Cryopreservation Media.

3. Biological Activity

This cell line has validated for cellular response to stimulation with GLP-1.

Mycoplasma testing

The cell line has been screened using the PCR-based Venor™GeM Mycoplasma Detection kit (Minerva) to confirm the absence of Mycoplasma species.

Storage

Immediately upon receipt, store in liquid nitrogen.

4. Materials to Be Supplied by the User

Recommended Reagents

Dulbecco’s Modified Eagle’s Medium - high glucose (D6429 Sigma-Aldrich)

MEM Non-essential Amino Acid Solution (100×) (M7145 Sigma-Aldrich)

Fetal bovine serum (FBS)

DPBS with calcium and magnesium (Sigma Aldrich D8662)

Opti-MEM (Life technologies 31985-070)

5. Supplies and Equipment

96-well assay plate

Tissue culture flasks

Class II biological safety cabinet

Hemacytometer

Incubator humidified 37°C, 5% CO_2

Inverted microscope

Fluorimeter

6. EXPERIMENTAL PROTOCOL FOR FLUORIMETER ASSAY

- Thaw cells rapidly. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Do not allow sample to warm to 37ºC. Cryovials should be cool to the touch when removed from bath. Passive thaw is not recommended.

- Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field.

- Immediately transfer contents of vial to a 15 mL tube

- Drop wise add warm media until the 8 ml demarcation. Gently invert the tube to distribute contents.

- Centrifuge at 300g for 5 minutes. Remove supernatant and resuspend cell pellet in warm medium (20 ml)

- Dispense the contents of the tube into a 96 well plate. Approximately a seeding density of 30.000 cells per well in 96-well plates (200 µl per well).

- Incubate in a humidified 37ºC / 5% CO_2 incubator overnight.

- Prepare *Assay medium* and warm prior to treat cells: Opti-MEM (*Ref. Life Technologies: 51985-026*)

- Remove *thawing medium* and replenish with *Assay Medium*

- Add reference compounds or test compounds dissolve in Opti-MEM. Add Opti-MEM including the vehicle of the compounds to unstimulated control wells. Set up each treatment for at least triplicate.

- Incubate cells in a humidified 37ºC / 5% CO^2 for 24 hours.

- Remove the assay medium and replace it by 100µl of DPBS with calcium and magnesium. Read the plate using the appropriate filter for the turboFP650 protein fluorescent signal, with excitation and emission peaks at 592 nm and 650 nm, respectively

- Data Analysis: Substrate average background fluorescence (NECA-free control wells) from fluorescence reading of all wells.

7. EXPERIMENTAL DESIGN FOR IMAGE ANALYSIS ASSAY

-Thaw cells rapidly. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Do not allow sample to warm to 37ºC. Cryovials should be cool to the touch when removed from bath. Passive thaw is not recommended.

-Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field.

-Immediately transfer contents of vial to a 15 mL tube

-Drop wise add warm media until the 8 ml demarcation. Gently invert the tube to distribute contents.

-Centrifuge at 300g for 5 minutes. Remove supernatant and resuspend cell pellet in warm medium (20 ml)

-Dispense the contents of the tube into a 96 well plate. Approximately a seeding density of 20.000 cells per well in 96-well plates (200 µl per well).

-Incubate in a humidified 37ºC / 5% CO_2 incubator overnight.

-Prepare *Assay medium* and warm prior to treat cells: Opti-MEM (*Ref. Life Technologies: 51985-026*)

-Remove *thawing medium* and replenish with *Assay Medium*.

-Add reference compounds or test compounds. Add Opti-MEM including the vehicle of the compounds to unstimulated control wells. Set up each treatment for at least triplicate.

-Incubate cells in a humidified 37ºC / 5% CO^2 for 24 hours.

-Fix the cells using formaldehyde solution (3.7 wt. %, 15 minutes). After fixation, permeabilize the cells using permeabilization solution (0,3% wt.%, 3 min). Nuclei were stained using DAPI (2 ng/ml) and the fluorescence was measured using a BD Pathway 855 High-Content Bioimager from Becton Dickinson. Nuclei number was measured by quantifying DAPI-stained ROIs (region of interest), whereas Nomad including Vesicle number was measured by quantifying FP650-containing ROIs. To detect DAPI, 380/10 and 84101 filters for excitation and emission were used respectively. And for the detection of Nomad-FP650 vesicles, 548/20 and 570LP filters were used.