

Assay Protocol

CB1_{MTX}Nomad HEK293 cell line

1. Introduction

Nomad multiplex assays are used to investigate both β -arrestin and Ca^{++} signaling pathways as high-throughput screening tools in drug discovery. _{MTX}Nomad HEK293 cell line is a clonal derivative of HEK293 cells stably expressing two Nomad Biosensors, Green Fluorescent β -arrestin Nomad Biosensor and Red Fluorescent cAMP Nomad Biosensor. Cannabinoid Receptor type 1, also known as CNR1 Receptor (CB1) has been cloned into _{MTX}Nomad HEK293 cell line. The resultant Cell line called, CB1_{MTX}Nomad HEK293 cell line is designed for High throughput screening (HTS) analysis of the receptor response that results in a cellular cAMP and β -arrestin signaling pathway regulation. The activation of these signaling pathways mediated by CB1 Receptor leads to a change in the cellular localization of the Nomad biosensors and to an increment in the intensity of the biosensor's fluorescent signals.

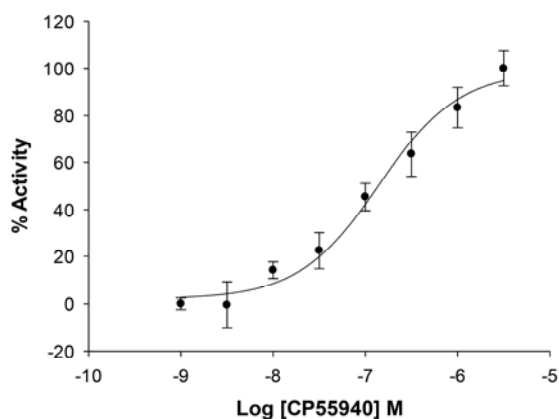
2. Product Components and Storage Conditions

Product: CB1_{MTX}Nomad Cell Line

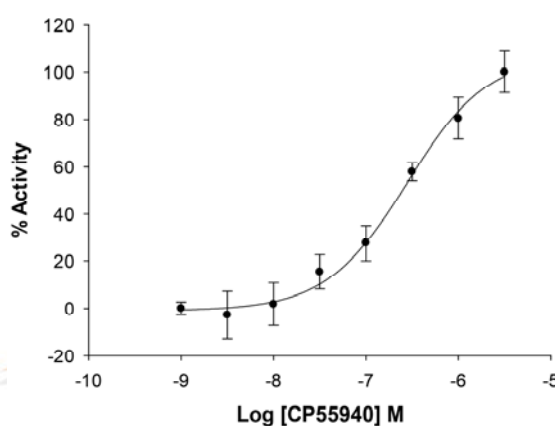
Size: 2 vials 3×10^6 cells in Freezing Media (Cryostor CS10).

3. Biological Activity

This cell line is validated for cellular response to stimulation with CP55940.



Arrestin-Nomad (tGFP):
Z': 0,72
Ec50: $1,40 \times 10^{-7}$ M



cAMP-Nomad (FP650):
Z': 0,69
Ec50: $2,25 \times 10^{-7}$ M

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

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

Hemocytometer

Incubator humidified 37°C, 5% CO₂

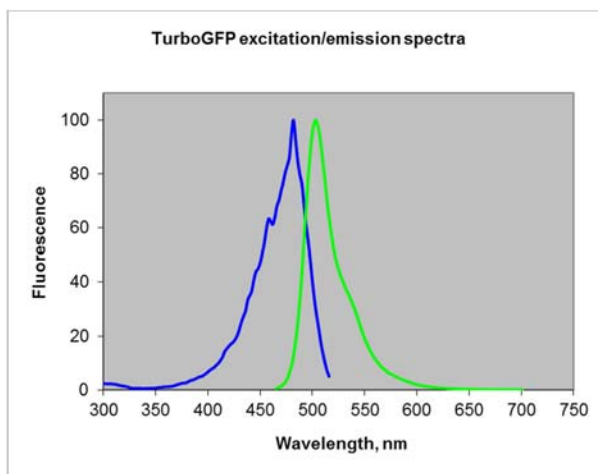
Inverted microscope

Fluorimeter

6. Experimental protocol for fluorimeter assay

- Seed density of 30.000 cells per well in 96-well plates (200 μ l per well).
- Incubate in a humidified 37°C / 5% CO₂ 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 37°C / 5% CO₂ for 24 hours.
- Remove the assay medium and replace it by 100 μ l of DPBS with calcium and magnesium. Read the plate using the appropriate filters:
 - For the cAMP Nomad biosensor fluorescent signal: excitation and emission peaks at 592 nm and 650 nm, respectively
 - For the Arrestin Nomad biosensor fluorescent signal: excitation and emission peaks at 482 nm and 502 nm, respectively
- Data Analysis: Substrate average background fluorescence (Agonist-free control wells) from fluorescence reading of all wells.

β -Arrestin Biosensor Spectra



cAMP-Nomad Biosensor Spectra

