NOMAD CELL LINES

icAMPNOMAD M4

Experimental Assay

1. Introduction

Nomad cell lines are a reliable system for studying G protein-coupled receptor (GPCR) signaling in living cells.

Optimized for the integration into High Content Screening (HCS) and High Throughput Screening (HTS) workflows, icAMPNomad-M4 Receptor Cell Line stably express red icAMPNomad Biosensor along with the Cholinergic receptor muscarinic 4 (M4).

2. Product components and recommended storage conditions

- M4 icAMPNomad (Innoprot P70545-02R)
- 2 vial 3x10^6cells in Freezing Media
- Storage: Immediately upon receipt, storage in liquid nitrogen

3. Biological Activity

- This cell line has been validated for cellular response to stimulation with oxotremorine (MedChem Express HY-101239) as agonist and atropine (Sigma-Aldrich A0132) as antagonist.
- Mycoplasma testing: The cell line has been screened using a Mycoplasma PCR Detection Kit (Abm G238) following manufacturer´s instructions.

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4. Recommended Reagents to Be Supplied by the User

- DMEM/Nutrient Mixture F-12 Ham (Sigma-Aldrich D8437)
- Fetal bovine serum (FBS) (Sigma-Aldrich F7524)
- DPBS with calcium and magnesium (Sigma Aldrich D8662)
- Opti-MEM - Glutamax (ThermoScientific 51985-026)
- Greiner CELLSTAR® 96 well plates flat bottom black polystyrene wells (with micro-clear bottom) (Greiner M0562-32EA)
- Formaldehyde Solution (Sigma Aldrich F1635)
- Triton™ X-100 (Sigma Aldrich T8787)
- Hoechst 33342 (ThermoFisher H1399)
- DAPI (Sigma Aldrich D9542)

5. Recommended Equipments

- Class II biological safety cabinet
- Hemacytometer / Cell counter
- Incubator humidified 37°C, 5% CO_2
- Inverted microscope
- Fluorimeter / Image analysis: Appropriate filter for the turboFP650 protein fluorescent signal, with excitation an emission peaks at 592 nm and 650 nm, respectively.

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6. Fluorimeter Assay: Experimental Protocol

- Day 1. Thaw Nomad cell line (3x10^6 cells per T25).
- Day 2. Maintain cells in DMEM-F12 supplemented with 10% FBS at 37 °C in a humidified 5% CO_2 atmosphere.
- Day 3. Plate cells at a concentration of 25,000 cells/plate in a 96-well plate and maintain them in DMEM-F12 medium supplemented with 10% FBS during 24h at 37 °C in a humidified 5% CO_2.
- Day 4. Incubate cells with the test compounds diluted in OptiMEM O/N.
- Day 5. Replace the medium with 100 µl PBS to perform the fluorescence intensity acquisition.
- Data Analysis: Substrate average background fluorescence (compound-free control wells) from total fluorescence acquired data.

7. Image Assay: Experimental Protocol

- Day 1. Thaw Nomad cell line (3x10^6 cells per T25).
- Day 2. Maintain cells in DMEM-F12 supplemented with 10% FBS at 37 °C in a humidified 5% CO_2 atmosphere.
- Day 3. Plate cells at a concentration of 25,000 cells/plate in a 96-well plate and maintain them in DMEM medium supplemented with 10% FBS during 24h at 37 °C in a humidified 5% CO2.
- Day 4. Incubate cells with the test compounds diluted in OptiMEM O/N.
- Day 5. ***In vivo* assay**. Add Hoechst diluted in OptiMEM to each well at a final concentration of 10-20 mg/ml without removing the overnight media (OptiMEM + compounds). Incubate 20-30 min at 37 °C in a humidified 5% CO_2 atmosphere. Replace the medium with 100 µl PBS to perform the fluorescence image acquisition.
- Day 5. **Fixed-Cell Imaging.** Fix the cells using formaldehyde solution (3.7 wt. %, 15 min). After fixation, permeabilize the cells using Triton X-100 diluted in PBS (0,03% wt.%, 3 min). Stain nuclei using DAPI at a final concentration of 2 ng/ml. Replace the medium with 100 µl PBS to perform the fluorescence image acquisition.
- Nomad signaling can be analyzed by fluorescence intensity or vesicle number count.

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