NOMAD CELL LINES

cAMPNOMAD ADORA2B

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, cAMPNomad-ADORA2B cell line stably express red cAMPNomad Biosensor along with the Adenosine A2B Receptor (ADORA2B).

2. Product components and recommended storage conditions

- ADORA2B $_{cAMP}$Nomad (Innoprot P70511)
- 1 vial 3x10^{6}cells 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 NECA (Tocris 1691) as agonist and PSB-603 (Sigma-Aldrich SML1983) as antagonist.
- Mycoplasma testing: The cell line has been screened using a Mycoplasma PCR Detection Kit (Abm G238) following manufacturer´s instructions.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edificio 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

innoprot cell based solutions

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 turboGFP protein fluorescent signal, with excitation an emission peaks at 592 nm and 650 nm, respectively.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

innoprot cell based solutions

NOMAD CELL LINES

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.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

innoprot cell based solutions