

# Experimental Assay

## DRD<sub>3</sub> cAMP Nomad U2OS cell line

### \* 1. Introduction

Nomad assays are valuable tools for conducting high-throughput screening in drug discovery. Nomad Biosensors are engineered to measure the intracellular dynamics of second messengers such as cAMP, Ca<sup>2+</sup>, DAG and the β-arrestin signaling.

Prior to GPCR activation, Nomad biosensors are localized in the plasma membrane. Upon ligand binding, the sensors undergo a conformational change that leads to an increase in the fluorescence intensity and their re-localization in the vesicular trafficking of the cells.

### \* 2. Product components and recommended storage conditions

- DRD<sub>3</sub> cAMP Nomad (Innoprot P70719-G)
- 2 vials 3x10<sup>6</sup> 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 Dopamine (Sigma-Aldrich H8502)
- Mycoplasma testing: The cell line has been screened using a Mycoplasma PCR Detection Kit (Abm G238) following manufacturer's instructions.

## \* 4. Recommended Reagents to Be Supplied by the User

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- 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 (Life technologies51985-026)
- 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 Equipment

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- Class II biological safety cabinet
- Hemacytometer / Cell counter
- Incubator humidified 37°C, 5% CO<sub>2</sub>
- 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.



## 6. Fluorimeter Assay: Experimental Protocol

- Day 1. Thaw Nomad cell line ( $3 \times 10^6$  cells per T25).
- Day 2. Maintain cells in DMEM-F12 supplemented with 10% FBS at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere.
- Day 3. Plate cells at a concentration of 22,000 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<sub>2</sub>.
- 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 ( $3 \times 10^6$  cells per T25).
- Day 2. Maintain cells in DMEM-F12 supplemented with 10% FBS at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere.
- Day 3. Plate cells at a concentration of 22,000 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% CO<sub>2</sub>.
- 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 µg/ml without removing the overnight media (OptiMEM + compounds). Incubate 20-30 min at 37 °C in a humidified 5% CO<sub>2</sub> 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.