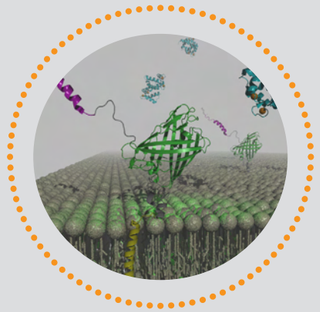
NEW GENERATION OF BIOSENSORS FOR HCS

Innoprot has developed a novel family of biosensors for measuring GPCR activity in living cells using the same fluorescent backbone. Each biosensor of the family co-expressed in a cell line with a GPCR, provides an innovative and sensitive research tool for studying the molecular mechanism and kinetics of GPCR activation. Nomad biosensors enable the measurement of second messenger concentration changes involved in GPCR activation. An increase in the second messenger concentration leads to a change in the structural folding of Nomad biosensor that promotes its cellular relocation. The molecular structure of Nomad biosensors comprises: a membrane localization peptide, a second messenger transduction protein binding peptide, a reticulum retention signal and a fluorescent peptide. The second messenger transduction protein binding peptide could be replaced depending on the second messenger involved in the GPCR activation pathway, resulting three different versions of Nomad biosensors: cAMP, calcium & DAG.



Advantages of Nomad™ Biosensors

- Different second messengers application
- Assays in living cells to study GPCR kinetics
- High sensitivity in robust assays
- Economic assay for cell processing and data analysis without any additional reagent
- Stable expression of the biosensor
- Non-tagged GPCRs

Applications

- High Content Screening for GPCR activity in living cells
- Live cell imaging to follow cellular effect kinetics of GPCR activation
- Ideal for high throughput screens based in fluorescence

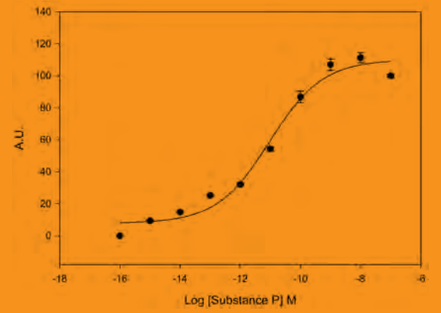
Proof of Concept

Nomad Biosensors have been validated by co-expressing them with several GPCRs in U2OS cell line. Upon receptor activation using their respective agonists, the activity was easily quantified by image analysis of cytoplasmic granularity changes following their corresponding second messenger increase. Nomad biosensors also provided a sensitive method for high throughput screening of drug libraries to identify compounds that modulate GPCR or any receptor that induces changes in second messengers levels.

1

Ca^{++} Nomad biosensor:

Measurement of calcium in living cells within a broad dynamic range of physiological concentrations of this second messenger.

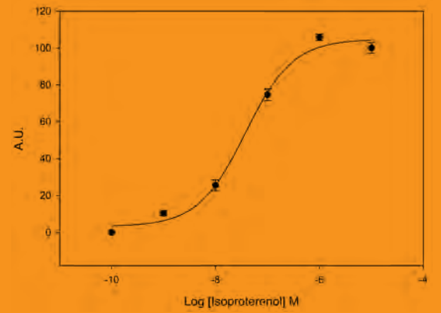


Concentration response curve for CCK octapeptide sulfated in CCKAR cell line co-transfected with Ca^{++} Nomad Biosensor. Cells were treated with 11 log dilution series (n=5). The Ec50 was ˜ 3.55 x 10-9 M after 24 h treatment with the agonist. The assay was validated for High Content Screening with an average of Z´=0.80+/- 0.02

2

cAMP Nomad biosensor:

Measurement of cAMP in living cells within a broad dynamic range of physiological concentrations of this second messenger.

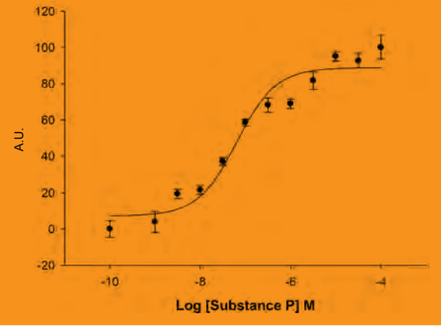


Concentration response curve for PACA-38 in VIPR cell line co-transfected with cAMP Nomad biosensor. Cells were treated with 11 log dilution series (n=5). The Ec50 for PACA-38 was ˜ 3.60 x 10-7 M after a 48h treatment with the agonist. The assay was validated for High Content Screening with an average of Z´=0.80+/- 0.02.

3

DAG Nomad biosensor:

Measurement of DAG in living cells within a broad dynamic range of physiological concentrations of this second messenger.



Concentration response curve for Substance P in TACR3 cell line co-transfected with DAG Nomad biosensor. Cells were treated with 11 log dilutuion series (n= 5). The Ec50 for Substance P was 6.46 x 10-8 M after a 48h treatment with the agonist. The assay was validated for High Content Screening with an average of Z´= 0.67+/- 0.02