NOMAD Biosensors

Multiplex screening for GPCRs

Innoprot has developed a new biosensor technology (NOMAD) for screening compounds against GPCR targets in functional cell-based assays. Amongst NOMAD's many advantages, you can easily evaluate G-protein and β-arrestin modulation in the same assay, hence allowing **biased activity studies**. Five NOMAD biosensors are available for detection of Ca2+, cAMP, DAG, RhoGTP or β-arrestin recruitment. Any combination of NOMAD biosensors can be co-expressed with the target GPCR, according to screening objectives. NOMAD assays are highly robust and well suited for high throughput screening (HTS) applications.

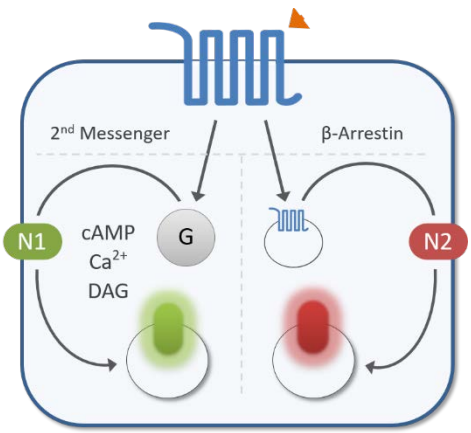
Key Features

- G-protein and β-arrestin signaling modulation can be measured in the same assay
- No need to label the target GPCR
- Direct measurement of Ca2+, cAMP, RhoGTP or DAG flux, or β-arrestin recruitment
- Assay have high Z' scores and low background
- Adapted to HTS (384 well plate format)
- Compound activity can be measured by either fluorescence or biosensor translocation
- Low running cost
  - No dyes or special reagents needed
  - Simple protocol and minimal hands-on time
  - Measure using standard lab equipment

Technology Access

Innoprot offers over 40 off-the-shelf NOMAD cell lines, and can also custom develop NOMAD cell lines as a service. NOMAD cell lines can be transferred to the client or used in contract research projects at Innoprot. Time to delivery of a new assay in 384 well plate-adapted format is typically about 3-4 months. Please contact us for more information –we would be pleased to discuss how NOMAD may help accelerate your research program.

How NOMAD works



Example of a cell expressing two Nomad biosensors (N1 and N2). Stimulation of the target GPCR triggers G protein and / or β-Arrestin-mediated signaling pathways. An increase in intracellular levels of 2nd messenger (choice of cAMP, RhoGTP, Ca2+ or DAG) causes N1 to internalize and emit green fluorescence. On the other hand, β-Arrestin recruitment followed by GPCR internalization causes N2 to internalize and emit red fluorescence. Modulation of the G protein and β-Arrestin signaling pathways by test compounds can thus be evaluated in a single assay.

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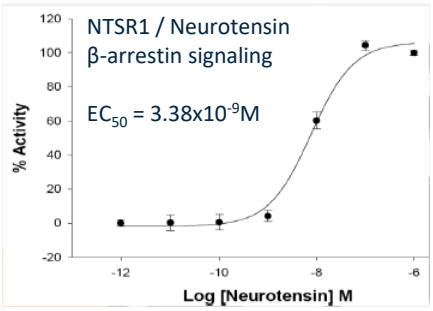
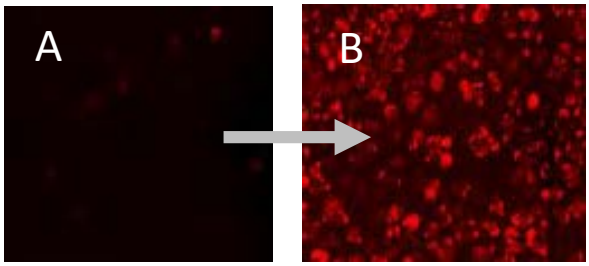
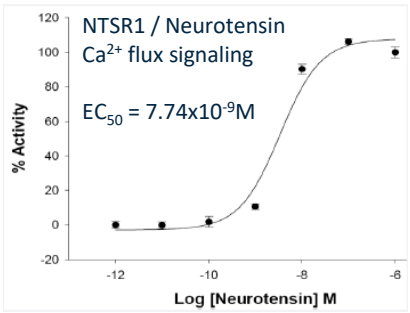
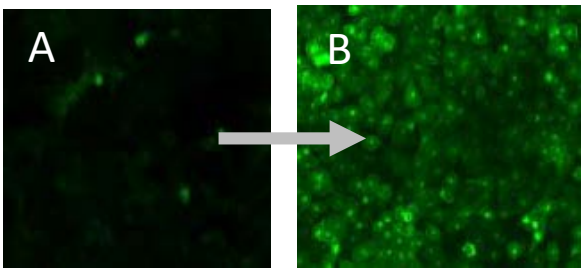
Biased activity study - sample data









Measuring β-Arrestin and Ca^{2+} -mediated signaling activity in a single assay: *A cell line expressing Neurotensin Receptor 1 (NTSR1), a Nomad β-Arrestin sensor (red) and a Nomad Ca^{2+} sensor (green) was stimulated with neurotensin. This led to an increase of similar magnitude in both β-Arrestin and Ca^{2+} -mediated signaling activity. Panels A and B show unstimulated cells and cell stimulated with 1µM neurotensin, respectively. Calculated EC50 values agree well with the literature, and the measurements were robust as indicated by Z-scores of 0.84 and 0.9 for the β-arrestin and Ca^{2+} assays respectively.*

Off-the-shelf NOMAD cell lines

Sensor	GPCR
cAMP flux	ADORA2B
	ADRB2
	CRHR2
	FSHR
	GLP1R
	GLP2R
	LHCGR
	M4R
	MCR3
	PAC1R
	VIPR1
	VIPR2

Sensor	GPCR
Ca2+ flux	BB2
	CCK1
	CCK2
	M5
	NK1
	NK3
DAG flux	M5
b-Arrestin recrutiment	NTSR1
	NK1R
Multiplex Ca^{++}-AMP	ENDRB
	NK2

Sensor	GPCR
Multiplex Arrestin-Ca^{++}	NTSR1
	NK1R
Arrestin-Ca^{++}	PAR2

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