

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**. Four NOMAD biosensors are available for detection of Ca^{2+} , cAMP, DAG 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.

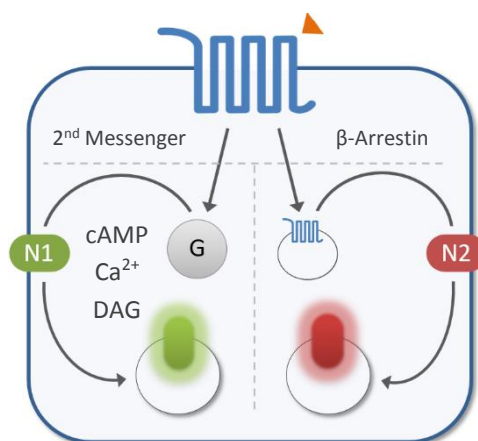
Key Features

- G-protein and β -arrestin signaling modulation can be measured in the same assay
- No need to label the target GPCR
- Direct measurement of Ca^{2+} , cAMP or DAG flux, or β -arrestin recruitment
- Assays have high Z' scores and low background
- Adapted to HTS (384 well plate format)
- Compound activity can be measured by fluorescence intensity or sensor 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 2-4 months. Please contact us for more information –we would be pleased to discuss how NOMAD may help accelerate your research program.

How NOMAD works

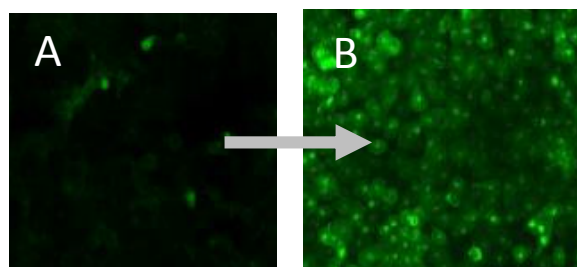
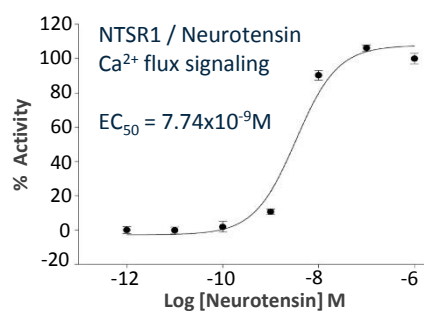
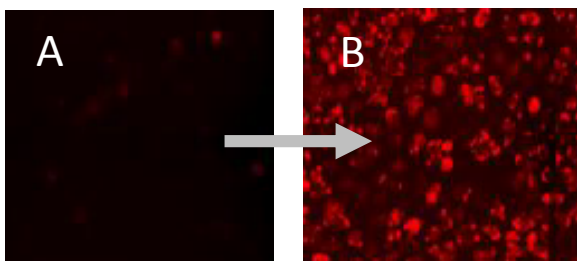
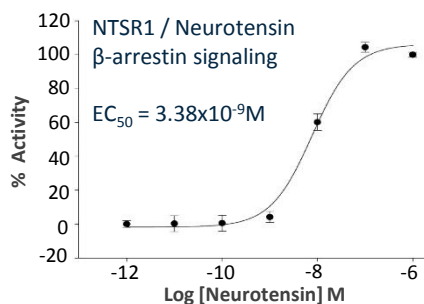


Example of a cell expressing two Nomad biosensors (N1 and N2). Stimulation of the target GPCR triggers G protein and / or β -Arrestin-mediated signaling pathways. An increase in intracellular levels of 2nd messenger (choice of cAMP, Ca^{2+} or DAG) causes N1 to internalize and emit green fluorescence. 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 $1 \mu M$ neurotensin, respectively. Calculated EC_{50} 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	Sensor	GPCR	Sensor	GPCR	Sensor	GPCR
Multiplex Arrestin- Ca^{++}	ADRA1A	Nomad-cAMP	ADORA1	Nomad-cAMP	FSHR	Nomad-DAG	ADRA1A
	ADRA1B		ADORA2A		GLP1R		ADRA1B
	BDKR2		ADORA2B		GLP2R		CCKAR
	CCKBR		ADORA3		LHR		ETBR
	GRPR		ADRB2		M4		GRPR
	HRH1		ADRB3		PACAPR1	M5	
	NK1R		CALCR		TSHR	Nomad- Ca^{++}	CCKBR
	NTSR1		CB1		VIPR1		GNRHR
	OXTR		DRD1		VIPR2		NK1
	PAR2		DRD2		Multiplex cAMP- Ca^{++}		NK2R
CB1	DRD5	NTSR1	NTSR1				