Nano-Glo® HiBiT Extracellular Detection System

Bioluminescent Detection of Cell-Surface Expressed Proteins

The Nano-Glo® HiBiT Extracellular Detection System quantitates cell surface or secreted protein expression in minutes using a simple add-mix-read assay format.

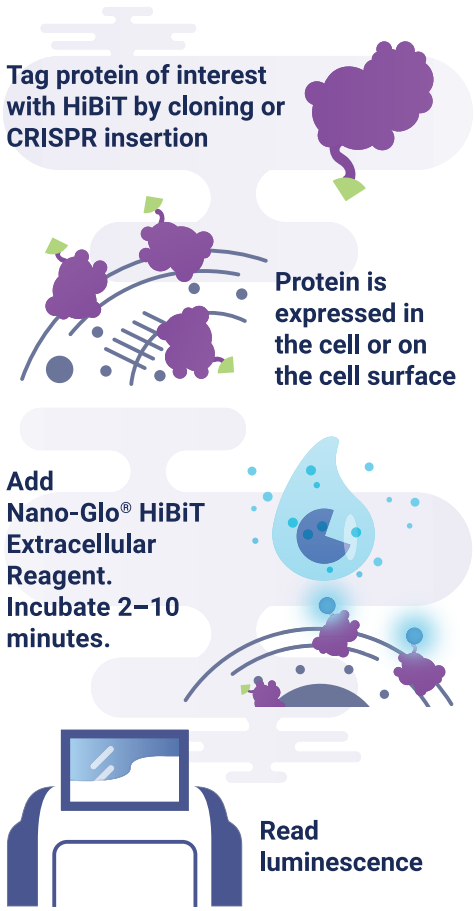
First, the 11-amino acid HiBiT peptide tag is added to the protein of interest using either traditional cloning or CRISPR/Cas9 genome editing approaches. Any proteins that are tagged with the HiBiT peptide and expressed outside of the cell can be specifically quantitated by adding the Nano-Glo® HiBiT Extracellular Detection Reagent.

The detection reagent provides luciferase substrate and the membrane-impermeable complementing polypeptide LgBiT, which spontaneously interacts with the HiBiT tag to reconstitute the bright, luminescent NanoBiT® enzyme. Luminescence is directly proportional to the amount of HiBiT-tagged protein present outside the cell over seven orders of magnitude, and the glow-type signal is stable for hours.

Simple, Senstive Method Monitors Extracellular Protein Abundance

The Nano-Glo® HiBiT Extracellular Detection System is a highly quantitative assay with significantly fewer processing steps than standard antibody-based detection methods. The assay can be completed in minutes and the broad linear dynamic range enables accurate quantification of tagged proteins regardless of expression level. Example applications include development of quantitative assays for:

- Receptor internalization
- Receptor recycling
- Protein or cytokine secretion
- Surface protein trafficking

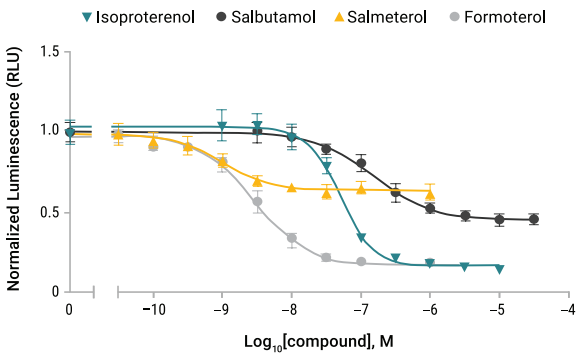


Nano-Glo® HiBiT Extracellular Detection System protocol.

Measures Receptor Internalization in Minutes

The Nano-Glo® HiBiT Extracellular Detection System makes it possible to develop simple, quantitative assays for receptor internalization that save time and eliminate the variability associated with antibody-based methods. The optimized detection reagent results in rapid equilibration with protein receptors. minimizing well-to-well variability and capturing rapidly changing biology. For example, in the figure to the right, we measured internalization of β2-adrenergic receptor (ADRB2) when exposed to four full and partial agonists.

Measure Potency and Extent of Receptor Internalization

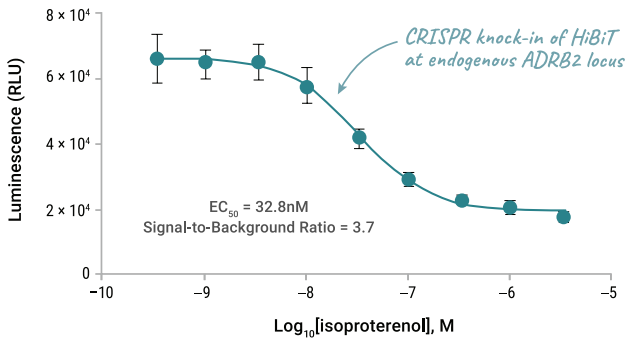


Agonist	EC_{50}	Percentage Receptor Remaining on Surface
Isoproterenol	50.9 nM	16%
Salbutamol	161 nM	45%
Salmeterol	1.04 nM	63%
Formoterol	2.92 nM	16%

Detects and Quantifies Endogenous Proteins

The small size of the 11 aa HiBiT tag enables simple cloning-free genome editing. Using CRISPR/Cas9, the HiBiT tag can be precisely inserted at endogenous loci. The Nano-Glo HiBiT Extracellular System provides significant new capabilities for studying the biology of endogenously regulated membrane receptors, reducing overexpression artifacts and maintaining proper stoichiometry with interacting proteins.

Internalization of Endogenously Expressed ADRB2



For more information about the Nano-Glo® HiBiT Extracellular Detection System, visit:
www.promega.com/HibitExtra

Ordering Information

Product	Size	Cat.#
Nano-Glo® HiBiT Extracellular Detection System	10ml	N2420
	100ml	N2421
	10 × 100ml	N2422

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