

NanoBRET™ Technology

*Investigate the Dynamics of
Protein:Protein Interactions in Live Cells*

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The NanoBRET™ PPI System represents a powerful and robust method for the investigation of protein:protein interactions (PPI) in live cells with applicability to high-throughput screenings (HTS). It enables dynamic measurements of protein interactions in real-time and it is used in both applied and academic research. Monitoring signaling pathway activity is one application of the NanoBRET™ PPI System.

Principle

The NanoBRET™ PPI System is based on the phenomenon of bioluminescence resonance energy transfer (BRET) in which energy is transferred from a donor luciferase to an acceptor fluorophore in a radiation-free manner, as these two components are brought into close proximity (< 10 nm). The resulting excitation of the fluorophore can be detected by light emission at longer wavelengths. The combination of the intensely bright NanoLuc® luciferase with the spectrally adjusted NanoBRET™ 618 fluorophore forms the centerpiece of the NanoBRET™ technology (see Figures 1 and 2). This combination allows the set up of homogeneous and robust protein interaction assays with an excellent signal-to-noise ratio.

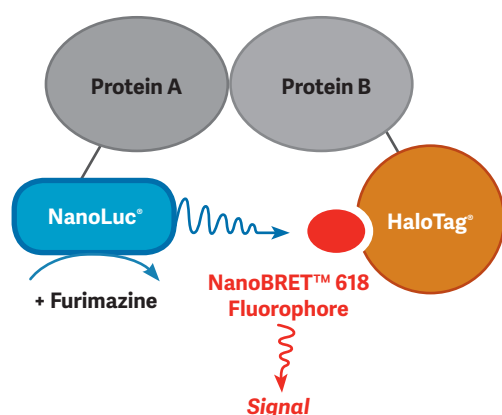


Figure 1: Measurement of protein:protein interactions in live cells with NanoBRET™ requires the expression of the interaction partners (A and B) as fusion proteins with NanoLuc® and HaloTag®. Upon interaction of protein A and protein B energy will be transferred from the NanoLuc® luciferase to the NanoBRET™ 618 fluorophore, which is covalently attached to the HaloTag® protein. Finally, the energy transfer results in acceptor fluorophore excitation and light emission.

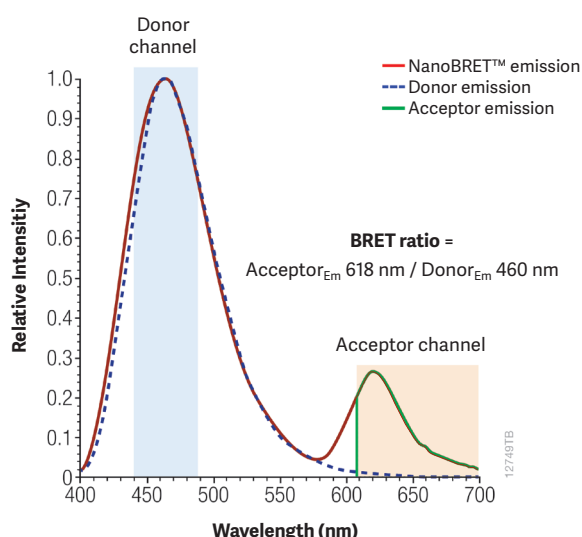


Figure 2: The large spectral separation of donor (NanoLuc®, Em_{max} : 460 nm) and acceptor signals (NanoBRET™ 618 fluorophore, Em_{max} : 618 nm) facilitates an improved signal-to-noise ratio. The signals are recorded in two separated channels: (1) Donor channel at 460 nm using a bandpass filter, and (2) acceptor channel at 610 nm using a longpass filter. The BRET ratio is determined by dividing the acceptor signal by the donor signal.

Features

Live cell assay: Measurement of protein:protein interactions in their native environment

Robust: Low assay variability and high reproducibility (high Z' factor)

Physiological expression level: Study induction and inhibition of protein interactions using full-length proteins expressed at physiologically relevant levels

High-throughput approved: 96- or 384-well plates

Ratiometric assay: No further normalization against cell number/protein content required

Sensitivity/linear range: Significantly improved compared to conventional BRET methods due to the wide spectral separation of donor and acceptor emission

Intrinsic negative control: No separate "donor-only" transfection required

Applications of NanoBRET™ in Live Cells

NanoBRET™ allows the measurement of protein:protein association and dissociation in live cells. Therefore, it is especially useful in experiments addressing the induction or inhibition of protein interactions.

Areas of Application

- Small molecule/off-target screening
- Peptide library screening
- Validation of *in vitro* data
- Kinetic measurements
- Dose-response measurements
- Reporter cell lines

Ligand-induced protein interaction as demonstrated for the recruitment of β -arrestin 2 to the vasopressin receptor 2

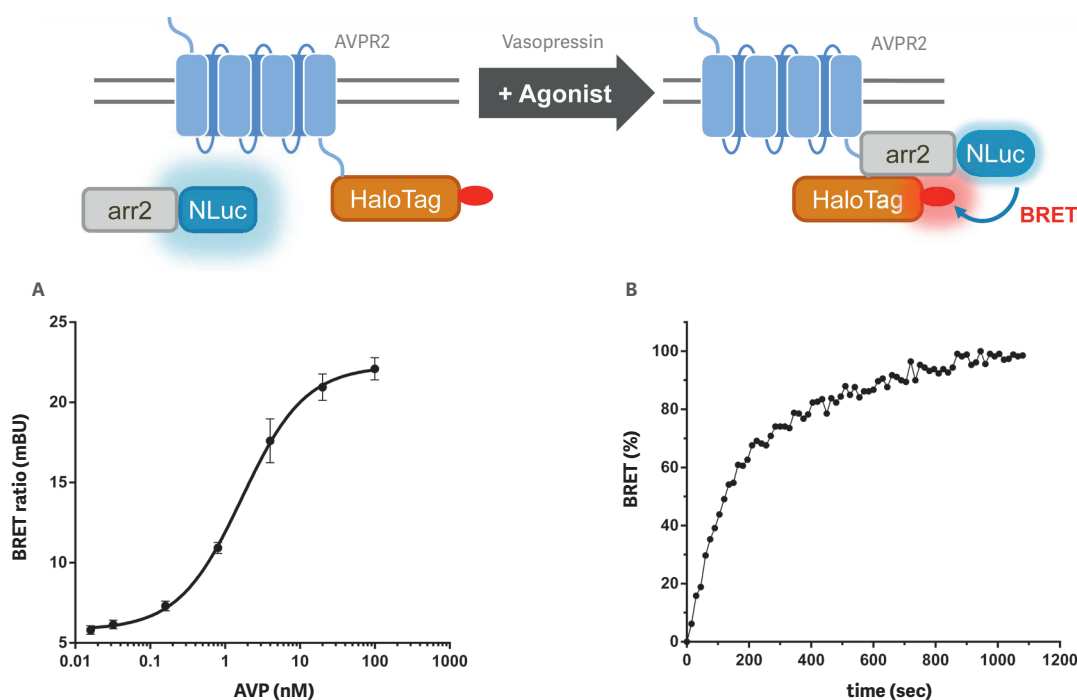


Figure 3: (A) Dose-dependent and (B) time-dependent recruitment of β -arrestin 2 (arr2) to the vasopressin receptor 2 (AVPR2) after addition of the peptide hormone arginine vasopressin (AVP).

Inhibition of p53:MDM2 interaction by Nutilin-3

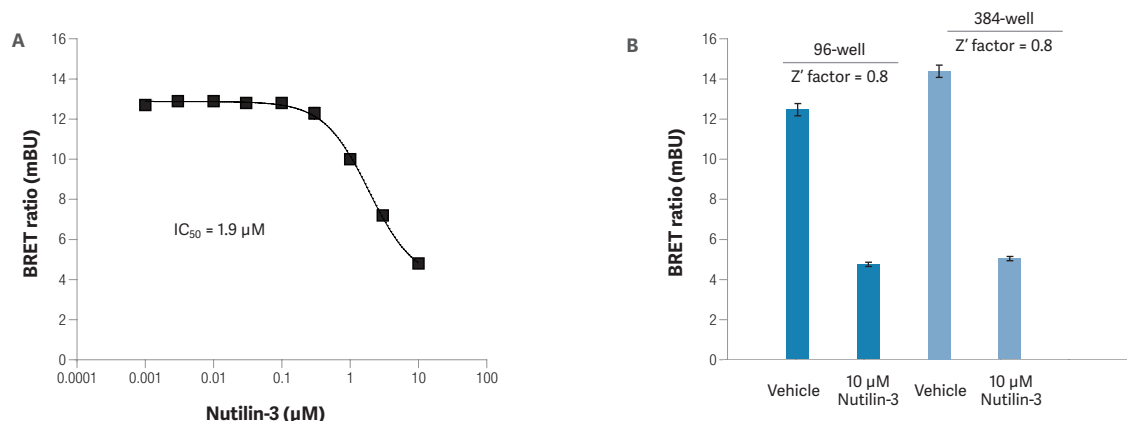


Figure 4: (A) Dose-dependent inhibition of p53:MDM2 interaction by Nutilin-3. (B) Effect of a single dose Nutilin-3 on the p53:MDM2 interaction in 96-well or 384-well-format with corresponding Z' factors.

Ready-to-use NanoBRET™ Protein Interaction Assays

Directly get started with our selection of ready-to-use protein interaction assays. The pre-built and validated assays contain experimentally optimized vector constructs, protocols and reagents for a variety of interesting biological targets.

- **Epigenetic protein assays**, e.g. bromodomain:histone interaction
- **Signal protein assays**, e.g. Kras/Braf
- **Kinase assays**, e.g. ERK/ELK
- **Transcription factor assays**, e.g. cMyc/Max
- **Membrane protein assays**, e.g. EGFR/GRB2
- **RNA binding protein assays**, e.g. hnRNP/hnRNP

Assays on the website

For a complete list of pre-built NanoBRET™ assays visit our Promega website:
www.promega.com/nanobret

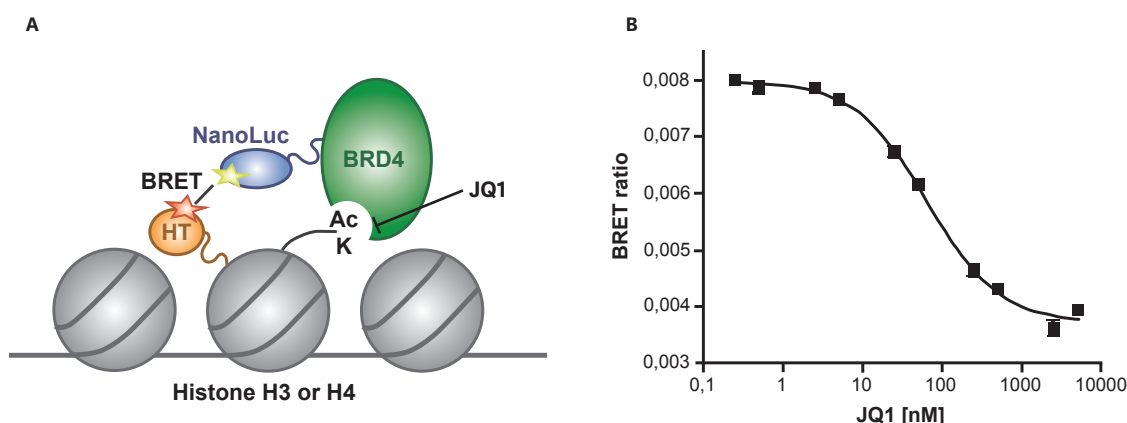


Figure 5: (A) Schematic illustration of bromodomain:histone interaction using BRD4:H3 or BRD4:H4. (B) Representative data for dose-dependent inhibition of bromodomain 4:histone 3.3 interaction by the small molecule bromodomain inhibitor JQ1.

"Ready-to-use" NanoBRET™ Assays

	Size	Cat.No.
NanoBRET™ BRD4/Histone H3.3 Interaction Assay	1x each*	N1830
NanoBRET™ BRD4/Histone H4 Interaction Assay	1x each*	N1890
NanoBRET™ BRD9/Histone H3.3 Interaction Assay	1x each*	N1840
NanoBRET™ BRD9/Histone H4 Interaction Assay	1x each*	N1900
NanoBRET™ CBP/Histone H3.3 Interaction Assay	1x each*	N1850
NanoBRET™ BRPF1/Histone H3.3 Interaction Assay	1x each*	N1860
NanoBRET™ BRPF1/Histone H4 Interaction Assay	1x each*	N1910
NanoBRET™ cMyc/MAX Interaction Assay	1x each*	N1870
NanoBRET™ KRas/Braf Interaction Assay	1x each*	N1880
NanoBRET™ PPI Control Pair (p53,MDM2)	1x each*	N1641

* All ready-to-use NanoBRET™ assays contain, in addition to the optimized and validated vector constructs, the control pair p53/MDM2 and reagents for 200 assays (96-well). A complete list of the ready-to-use NanoBRET™ assays can be found: www.promega.com/nanobret

NanoBRET™ PPI Starter Systems

The NanoBRET™ PPI Starter Systems provide the expression vectors required to generate N- and C-terminal NanoLuc® luciferase and HaloTag® protein fusions to target proteins. Additionally, the starter systems contain detection reagents for 200 assays (96-well) / 500 assays (384-well) and a NanoBRET™ positive control pair (p53-HaloTag® and NanoLuc®-MDM2).

Save time & find your
Flexi®-compatible "ready-to-use"
ORF-clone in FindMyGene™!
www.promega.com/findmygene

NanoBRET™ PPI Flexi® Starter System

Fast – Efficient – High Fidelity Cloning Method

The NanoBRET™ PPI Flexi® Starter System is composed of Flexi® vectors for the rapid and easy generation of required expression constructs. The system provides expression vectors for the generation of N- and C-terminal NanoLuc® and HaloTag® protein fusions using the Flexi® Vector Cloning System. The Flexi® system is a directional cloning method based on the two rare-cutting restriction enzymes, SgfI and PmeI. Prerequisite for the application of Flexi is that neither SgfI nor PmeI cut in the Open Reading Frame (ORF) of interest. In general the workflow starts with an initial PCR of the ORF of interest introducing SgfI and PmeI restriction sites. The resulting fragment is introduced into the pFN21A HaloTag® vector which serves as a shuttle vector for the rapid and easy generation of the remaining N- and C-terminal NanoLuc® and HaloTag® fusions. The Flexi® Vector Cloning System provides a high-fidelity way to transfer protein-coding regions between Flexi® Vectors without the need to resequence. The Find My Gene™ service contains many ORF clones already in Flexi® format for simple creation of protein fusions.

NanoBRET™ PPI MCS Starter System

The NanoBRET™ PPI MCS Starter System contains the required expression vectors for the generation of N- and C-terminal NanoLuc® and HaloTag® fusions to target proteins using traditional cloning via a multiple cloning site (MCS). Users of the MCS vectors should make sure that the insert is cloned in frame and that the respective start and stop codons are positioned properly.

NanoBRET™ PPI Starter Kits	Size	Cat.No.
NanoBRET™ PPI Flexi® Starter System	1x each*	N1821
N1321: pFN31K Nluc CMV-neo Flexi® Vector		
N1341: pFC32K Nluc CMV-neo Flexi® Vector		
G2821: pFN21A HaloTag® CMV Flexi® Vector		
G9661: pFC14K HaloTag® CMV Flexi® Vector		
N1641: NanoBRET™ PPI Control Pair (p53, MDM2)		
N1661: NanoBRET™ Nano-Glo® Detection System (200 assays, 96-well)		
NanoBRET™ PPI MCS Starter System	1x each*	N1811
N1351: pNLF1-N [CMV/Hygro] Vector		
N1361: pNLF1-C [CMV/Hygro] Vector		
G7721: pHTN HaloTag® CMV-neo Vector		
G7711: pHTC HaloTag® CMV-neo Vector		
N1641: NanoBRET™ PPI Control Pair (p53, MDM2)		
N1661: NanoBRET™ Nano-Glo® Detection System (200 assays, 96-well)		

NanoBRET™ Nano-Glo® Detection System

The NanoBRET™ Nano-Glo® Detection System provides the NanoBRET™ Nano-Glo® Substrate used by NanoLuc® luciferase to generate the donor signal and the HaloTag® NanoBRET™ 618 Ligand as fluorescent energy acceptor. The detection system is available in three different sizes.

NanoBRET™ Nano-Glo® Detection Reagents	Size	Cat.No.
NanoBRET™ Nano-Glo® Detection System	200 assays / 96-well plates 500 assays / 384-well plates	N1661
NanoBRET™ Nano-Glo® Detection System	1.000 assays / 96-well plates 2.500 assays / 384-well plates	N1662
NanoBRET™ Nano-Glo® Detection System	10.000 assays / 96-well plates 25.000 assays / 384-well plates	N1663

NanoBRET™ Assay Workflow

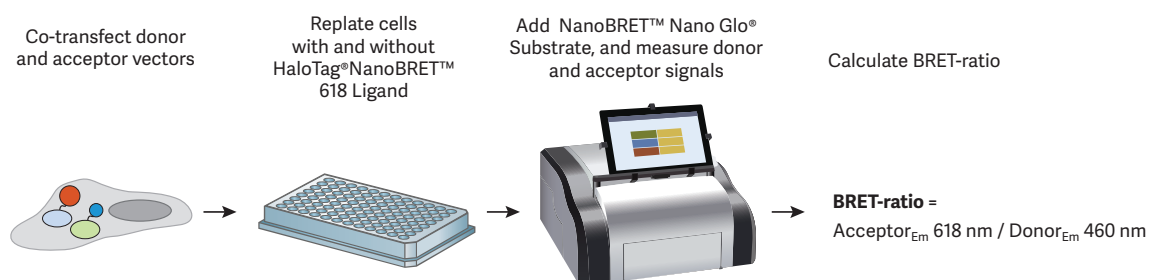


Figure 6: Schematic illustration of a NanoBRET™ PPI assay. In the first step the fusion constructs are transfected into live cells. Subsequently, the HaloTag® NanoBRET™ 618 Ligand is added directly to the cells during plating, whereas the addition is omitted to those cells serving as donor-only control. The NanoBRET™ Nano-Glo® Substrate is added to the sample just prior the measurement of donor and acceptor emission. Donor and acceptor signal are recorded with a BRET-compatible plate reader such as the GloMax® Discover System using a bandpass filter with 460 nm and a longpass filter with 610 nm. The acceptor signal is divided by the donor signal to calculate the BRET-ratio.

Literature NanoBRET™

Machleidt, T. *et al.* (2015) NanoBRET- A Novel BRET Platform for the Analysis of Protein:Protein Interactions. *ACS Chem Biol.* 2015 Jun 9.

Demont, E.H. *et al.* (2014) 1,3-Dimethyl Benzimidazolones Are Potent, Selective Inhibitors of the BRPF1 Bromodomain. *ACS Med Chem Lett.* 5(11):1190-5

Wang, J. *et al.* (2015) Activation of Rab8 guanine nucleotide exchange factor Rabin8 by ERK1/2 in response to EGF signaling. *Proc Natl Acad Sci.* 112 (1), 148–53.

Clark, P.G. *et al.* (2015) LP99: Discovery and Synthesis of the First Selective BRD7/9 Bromodomain Inhibitor. *Angew Chem Int Ed Engl.* 54(21):6217-21.

Detection of NanoBRET™

The detection of NanoBRET™ requires a BRET-capable plate reader equipped with two emission filters for the sequential measurement of filtered luminescence. A bandpass filter with 460 nm and a longpass filter with 610 nm are recommended to record the emission of the donor luciferase and the emission of the acceptor fluorophore.

The new multimode plate reader GloMax® Discover was optimized for the measurement of NanoBRET™. The instrument is equipped with a 450 nm/8 nm bandpass filter and a 610 nm longpass filter to record the donor and acceptor signal, respectively.



GloMax DISCOVER

A high-performance, easy-to-use multimode plate reader for luminescence, fluorescence, absorbance, BRET and FRET applications

GloMax® Discover allows the detection of luminescence, fluorescence, UV/Vis absorbance, BRET, FRET as well as the measurement of filtered luminescence. This high-performance instrument reads common plate formats 6-, 96- and 384-well with very high sensitivity and over a broad linear range. The system is easily operated by an integrated tablet-PC and can be incorporated in an automated workflow.

Various applications:

- Reporter gene assays
- Cell viability, cytotoxicity and apoptosis assays
- Kinetic measurements
- Multiplexing
- Assays for the detection of oxidative stress and cell metabolism
- ELISA
- BRET/FRET analysis

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