Interrogating Protein Interactions with the NanoBRET™ Assay

The number of protein interactions in human cells has been estimated to be upwards of **650,000** (Stumpf *et al.*, 2008[1]). Some of the processes in which protein interactions are involved include:

- Normal cellular processes
- Cellular response to stress
- Pathological Processes & Cancer
- ___ (fill in the blank with the interaction you're studying)

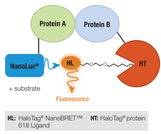
In your protein interactions studies, perhaps you've asked questions such as:

- Can I study my PPI within its natural biological context?
- Can I monitor PPI dynamics in real-time?
- Could inhibition of my PPI be important for disease treatments?
- Will my inhibitor compound be effective on full-length proteins in natural cellular context?
- How do mutations impact my PPI?

NanoBRET™ PPI Assays can help you answer these questions.

The new BRET-based protein:protein interaction (PPI) assay uses NanoLuc® Luciferase as the BRET energy donor and HaloTag® protein, labeled with the NanoBRET™ 618 fluorophore, as the energy acceptor to measure the interaction of specific protein pairs.

The improvements to this BRET system make it easier and more reliable.

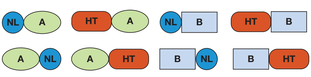


NL: HaloTag® NanoBRET™ 618 Ligand **HT:** HaloTag® protein

How do you design a NanoBRET™ Assay to monitor your protein interactions?

1 Generate Fusion Proteins

Prepare up to 8 possible clones containing combinations of protein A or protein B fused to NanoLuc (NL) and HaloTag (HT) at the amino (N) or carboxy (C) terminus of the protein.



Did you know?

Find My Gene™ makes this easy:

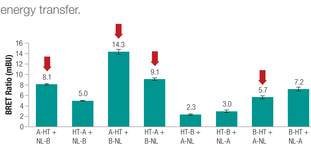
- Search a library of nearly 10,000 constructs to find genes encoding your protein of interest
- Receive your ORFs cloned into a Flexi® Vector that already has the HaloTag® protein appended to the N-terminus of the fusion protein
- Transfer an ORF to the remaining HaloTag® (C-terminal) and NanoLuc® (N- and C-terminal) fusion vectors using Flexi® cloning

FindMyGene provides a rapid, efficient, high-fidelity method to create fusions, so that there is no need to resequence. For more information, ***click here »***

Alternatively, NanoBRET™ Starter Systems are available, and provide the vectors required to create NanoLuc® Luciferase and HaloTag® protein fusions to target proteins of interest. For more information, ***click here »***

2 Test the Combinations

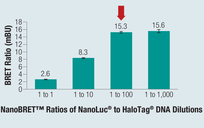
Using your clones, test these combinations to find the best energy transfer.



NanoBRET™ Ratios of PPI Combinations Tested

3 Optimize Transfection

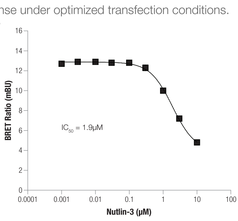
Find optimal donor to acceptor DNA ratio to minimize unbound donor and maximize dynamic range.



NanoBRET™ Ratios of NanoLuc® to HaloTag® DNA Dilutions

4 Validate

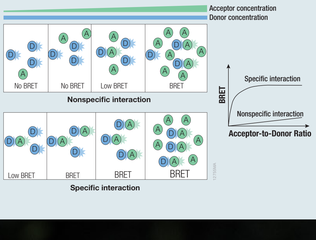
Use a known inhibitor or activator to test for a specific response under optimized transfection conditions.



Alternatively

Test Specificity

using a donor saturation assay.



For more information and other tools for your PPI studies visit:

www.promega.com/Try-NanoBRET

[1]Stumpf M.P.H. et al., (2008) Estimating the size of the human interactome. PNAS 105 (19) 6959-6964.