Application Note

MONITORING MOLECULAR INTERACTIONS USING THE PROMEGA NANOBRET™ PROTEIN:PROTEIN INTERACTION SYSTEM AND THE TRISTAR MULTIMODE MICROPLATE READERS

Abstract

Proteins perform a variety of functions in living cells and organisms and work together in a complex and coordinated way. The understanding of protein function requires analysis of protein interactions within the cellular context. The proximity-based BRET (Bioluminescence Resonance Energy Transfer) assay is an established technique to study protein:protein interactions, signal transduction pathways and receptors. The NanoBRET™ assay is a progression of this technology resulting in increased signal and lower background. For the detection of the signal, a suitable plate reader is required, such as the Tristar Multimode Microplate Readers developed by Berthold Technologies. In order to confirm the compatibility of the Promega NanoBRET™ System with the Tristar microplate readers, HEK293 cells were transiently transfected with the NanoBRET™ Positive Control Vector. It encodes a NanoLuc® and HaloTag® fusion protein that ensures energy transfer, which was detected using the NanoBRET™ Nano-Glo® Detection System. The results confirm that Tristar Multimode Readers are suitable devices for use with the Promega NanoBRET™ Protein:Protein Interaction System.

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Introduction

The NanoBRET™ System is an experimental set-up that enables the monitoring of protein interactions in live cells. The underlying principle is that of bioluminescence resonance energy transfer (BRET): The two proteins that are investigated are tethered to a NanoLuc® fusion protein as the energy donor and a fluorescently labelled HaloTag® fusion protein as the energy acceptor. In the presence of appropriate substrate, the NanoLuc® luciferase forms a luminescent product, which in turn excites the fluorescent protein acceptor if the two proteins are in close proximity. The optimized blue-shifted NanoLuc® donor paired with the red-shifted HaloTag® acceptor minimizes spectral overlap within the assay, resulting in an improved signal-to-background ratio when calculating the NanoBRET™ ratio.

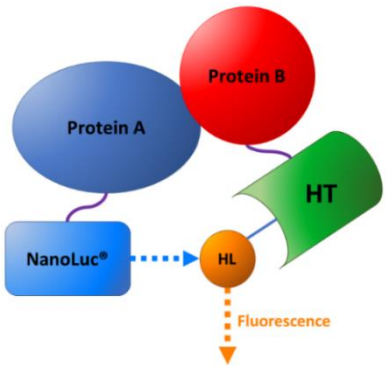

Figure 1: Basic principle underlying the NanoBRET™ protein:protein interaction assay. Protein A is tethered to a bioluminescent protein donor that excites the fluorescent acceptor fused to protein B if both proteins are in close proximity. HL: HaloTag® NanoBRET™ 618 ligand; HT: HaloTag® protein.

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Materials

- Berthold Technologies TriStar² S LB 942 Multimode Microplate Reader with extended spectral range luminescence module
- Promega NanoBRET™ positive control vector (catalogue no. N1581)
- Promega NanoBRET™ Nano-Glo® Detection System (catalogue no. N1661)
- FuGENE® HD Transfection Reagent (catalogue no. E2311)
- Sterile six-well plate with lid (Greiner 657160)
- White, opaque, sterile 96-well microplate (Berthold 51838)
- Human embryonic kidney (HEK) 293 cells and cell culture equipment and reagents
- Dulbecco's Modified Eagle's Medium (DMEM; Gibco catalogue no. 11995)
- Opti-MEM® I Reduced Serum Medium, no phenol red (Life Technologies catalogue no. 11058021)
- Fetal bovine serum (Sigma catalogue no. F0804)
- Penicillin/streptomycin solution (Sigma catalogue no. P4333)
- 0.05 % Trypsin/EDTA (Invitrogen catalogue no. 25300)
- DPBS (Invitrogen catalogue no. 14190)
- Dimethylsulfoxide (DMSO; Sigma catalogue no. 2650)

Instrument settings

- Excitation filter: none
- Emission filter donor: 460-70*
- Emission filter acceptor: 600-LP*
- Reading mode: luminescence
- Counting time: 1 s

* Included in NanoBRET™ filter package, ID-Number 63140.

Methods

Two days prior to the assay, cultured HEK 293 cells were trypsinized and diluted in cell culture medium to a final density of 4×10^5 cells/ml. Subsequently, 2 ml of cell suspension (800,000 cells) was plated into a well of a sterile six-well plate and incubated for 5 h at 37 °C and 5 % CO_2. 2 µg of Transfection Carrier DNA was mixed with 0.002 µg of the NanoBRET™ Positive Control Vector diluted in water and 100 µl of Opti-MEM® I Reduced Serum Medium was added to the transfection mixture. Next, 8 µl of FuGENE® HD Transfection Reagent was added and the mixture incubated at room temperature for 10 min. The transfection mixture was given to the cells, and these were incubated for 20 h at 37 °C and 5 % CO_2. The transfection mixture was subsequently removed and the cells rinsed with 1 ml of phosphate-buffered saline. The cells were trypsinized and resuspended in 2 ml of cell culture medium. Following centrifugation at 125 × g for 5 minutes, the supernatant was discarded and the cells resuspended in Opti-MEM® I Reduced Serum Medium with 4 % fetal bovine serum at a final density of 2×10^5 cells/ml. To half of the cells, 1 µl of 0.1 mM HaloTag® NanoBRET™ 618 Ligand per milliliter of cells (100 nM final concentration) was given, while the other half of cells were treated with 1 µl of DMSO per millilitre of cells (0.1 % DMSO final concentration) as no-ligand control. 100 µl of both cell suspensions were dispensed into separate wells of a sterile white 96-well microplate and the plate was incubated for 20 hours at 37 °C and 5 % CO_2. Subsequently, a 5x solution of NanoBRET™ Nano-Glo® Substrate in Opti-MEM® I Reduced Serum Medium was prepared and 25 µl given to each well. The plate was shaken for 30 sec and luminescence measured using the Tristar² S LB 942 Multimode Microplate Reader.

Results

To determine the corrected NanoBRET™ ratio, the luminescence signal for 3-4 wells each of HaloTag® NanoBRET™ 618 Ligand and DMSO as no ligand control was measured. The results are shown in Table 1.

Note: absolute values can be different depending on the specific Tristar reader used (for example, they will be lower using a Tristar 3 or 5 compared to the TriStar^2 S), but ratios obtained with the same filters and settings should be very similar.

	Donor Emission (mean RLU)	Acceptor Emission (mean RLU)	NanoBRET™ ratio (mBU)
Ligand	9,696,667	2,133,333	219.6
No ligand	15,600,000	94,980	6.1
Corrected NanoBRET™ ratio			**213.5**

Table 1: Luminescence values determined for the HaloTag® NanoBRET™ 618 Ligand and DMSO (no ligand). Data are mean values of 3-4 wells.

To account for donor-contributed background or bleedthrough, the NanoBRET™ ratio for the no-acceptor (DMSO) control is subtracted from the NanoBRET™ ratio calculated for the HaloTag® 618 Ligand. The resulting corrected NanoBRET™ ratio is 213.5 mBU. The Z' factor calculated from these results is 0.97, indicating a highly robust assay.
The assay was also tested using the standard luminescence module instead of the extended spectral range one; in this case the emission filter donor used was ID-Number 40272 (460-25), as it provided the best results (data not shown). Even though the NanoBRET™ ratio was lower than with the extended spectral range module (98.8 mBU instead of 213.5), the Z' factor was also excellent at 0.96.

These results demonstrate the high performance of the Tristar² S LB 942 Multimode Microplate Reader for the Promega NanoBRET™ Protein:Protein Interaction System.

Conclusions

A corrected NanoBRET™ ratio of 213.5 mBU and a Z' factor of 0.97 were obtained, confirming that the Berthold Technologies Tristar² S LB 942 Multidetection Microplate Reader is ideal for detection of the Promega NanoBRET™ Protein:Protein Interaction System. Both the standard and the extended spectral range luminescence modules are suitable for this application, but the extended spectral range one provides the best results.

As obtained ratios must be very similar using other microplate readers of the Tristar series, it can be concluded that all readers in the Tristar series are suitable for the measurement of the Promega NanoBRET™ Protein:Protein Interaction System.

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