APPLICATION NOTE

VALIDATION OF THE BERTHOLD TECHNOLOGIES MITHRAS² LB 943 MONOCHROMATOR MULTIMODE READER WITH THE PROMEGA NANOBRET™ PROTEIN:PROTEIN INTERACTION SYSTEM

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 Mithras² LB 943 Multimode Microplate Reader developed by Berthold Technologies. In order to confirm the compatibility of the Promega NanoBRET™ System with the Mithras² LB 943, HEK293 cells were transiently transfected with the NanoBRET™ Positive Control Vector, that 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 the Mithras² LB 943 is a suitable device 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.

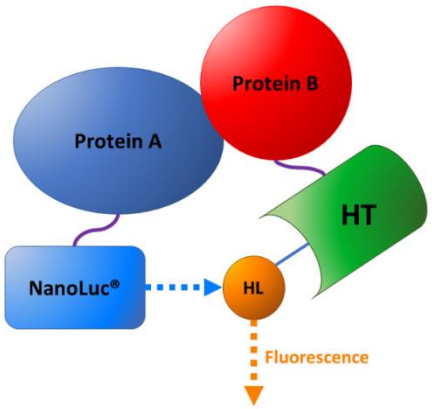

Figure 1: Basic principle underlying the NanoBRET™ protein:protein interaction assay. Protein A is tethered to a bioluminescent protein donor that excites the fluorescent acceptor fused to protein B if both proteins are in close proximity. HL: HaloTag® NanoBRET™ 618 ligand; HT: HaloTag® protein.

The Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader

The Mithras² LB 943 is a high-end microplate multimode reader based on monochromator technology with excellent performance. Characterized by its sensitivity and robustness, especially in luminescence and BRET measurements, the reader supports all important reading technologies:

- Luminescence
- BRET and BRET2
- Fluorescence
- FRET
- Fluorescence Polarisation
- UV/VIS absorbance
- AlphaScreen® and AlphaLISA®
- Time-resolved fluorescence
- TR-FRET

Mithras² additional features:

- Monochromator & filter technology
- Detectors: 2 low-noise PMTs (up to 850 nm) and ultra-low noise PMT operated in single photon counting mode, photo diode (200 – 1000 nm)
- Top & bottom reading
- Automatic plate height adjustment (Z optimization)
- Up to 4 JET injectors (98% accuracy & precision over entire volume range)
- All microplate formats up to 1536-well
- Shaking and incubation up to 45°C

Different reading technologies have their own demands on the optical system for optimal performance. In contrast to conventional multi-technology instruments, the Mithras multimode microplate reader has been designed with a proprietary optical system consisting of separate optical paths for different reading technologies (mDOPS). The separated light paths of the mDOPS optical system ensure that the needs for high sensitivity and a wide dynamic range are met for each reading technology. This results in high-end performance that typically can be achieved with dedicated instruments only.

Materials

- Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader
- 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 filters donor: 460-70*
- Emission filter acceptor: 600-LP*
- Reading mode: luminescence
- Counting time: 1 s

* Included in the NanoBRET™ RFID filter package (ID-Number 63141).

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 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 Mithras² LB 943 Monochromator Multimode 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.

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 264.9 mBU. The Z' factor calculated from these results is 0.93, indicating a highly robust assay. These results validate the performance of the Promega NanoBRET™ Protein:Protein Interaction System on the Mithras² LB 943 Monochromator Multimode Reader.

	Donor Emission (mean RLU)	**Acceptor Emission (mean RLU)**	**NanoBRET™ ratio (mBU)**
Ligand	8,399,785	2,259,139	272.1
No ligand	22,131,701	158,653	7.2
Corrected NanoBRET™ ratio			**264.9**

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 substracted from the NanoBRET™ ratio calculated for the HaloTag® 618 Ligand. The resulting corrected NanoBRET™ ratio is 264.9 mBU. The Z' factor calculated from these results is 0.93, indicating a highly robust assay. These results validate the performance of the Promega NanoBRET™ Protein:Protein Interaction System on the Mithras² LB 943 Monochromator Multimode Reader.

Conclusions

A corrected NanoBRET™ ratio of 264.9 mBU and a Z' factor of 0.93 were obtained, confirming that the Berthold Technologies Mithras² LB 943 Monochromator Multimode Reader is ideal for detection of the Promega NanoBRET™ Protein:Protein Interaction System.

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