

APPLICATION NOTE

NanoBRET Technology on the SpectraMax i3x Multi-Mode Microplate Reader

Cathy Olsen, PhD | Sr. Applications Scientist | Molecular Devices

Introduction

Protein-protein interactions (PPI) are vital to cellular function and signaling. Many methods have been employed to study PPI, including co-immunoprecipitation and pull-down assays, as well as fluorescence methods such as fluorescence resonance energy transfer (FRET). Because they rely on excitation of a sample by a laser or high-powered lamp, fluorescence methods are often prone to issues of photo-bleaching and background fluorescence, which can limit their usefulness. An alternative method, bioluminescence resonance energy transfer (BRET), uses a chemical reaction catalyzed by the enzyme luciferase to generate light. Because there is no optical excitation, BRET avoids problems posed by sample illumination with an external light source.

NanoBRET™ Technology, from Promega, uses NanoLuc® Luciferase as the BRET donor and HaloTag® protein labeled with the NanoBRET 618 fluorophore as acceptor. The red-shifted acceptor reduces overlap of the donor and acceptor emission spectra, enabling better signal to noise than other BRET methods that use *Renilla* luciferase as a donor and YFP or GFP as acceptor.

Here, we demonstrate validation of the SpectraMax® i3x Multi-Mode Microplate Reader for NanoBRET assays. The onboard luminescence detection mode may be used, or, for higher sensitivity, the reader can be equipped with a NanoBRET detection cartridge that offers a 4-fold improvement in limit of quantitation.

Benefits

- Study induction and inhibition of protein interactions with a physiologically relevant system
- Achieve increased signal and lower background compared to conventional BRET assays
- NanoBRET detection module for SpectraMax i3x offers extra sensitivity for assays that require it

Materials

- White 96-well plate
- 1X phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA)
- NanoBRET Control protein panel – 5 vials representing the following amounts of fractional occupancy:
 - NanoBRET Control Protein 1: 0%
 - NanoBRET Control Protein 2: 0.1%
 - NanoBRET Control Protein 3: 1%
 - NanoBRET Control Protein 4: 10%
 - NanoBRET Control Protein 5: 100%

- NanoBRET Nano-Glo Substrate (part of NanoBRET Detection System)
- SpectraMax i3x Multi-Mode Microplate Reader
- NanoBRET detection cartridge

Methods

An *in vitro* protocol provided by Promega for using a NanoBRET control protein was run to validate instrument setup and estimate the detection limit. A series of standards containing increasing amounts of NanoBRET active protein were plated in a 96-well solid white microplate for NanoBRET measurements. These measurements were used to generate linear regression calculations and limit of quantitation (LOQ) values used to gauge instrument performance.

The validation method was run on the SpectraMax i3x reader using either a custom NanoBRET detection cartridge or the onboard luminescence detection optics. Results for both are presented.

Assay method

- Dispense 50 μ L of control protein into triplicate wells of a 96-well plate.
- Prepare a 20 μ M solution of NanoBRET Nano-Glo Substrate in 1X PBS/0.1% BSA (250-fold dilution of 5 mM stock reagent).
- Add 50 μ L of substrate to each well for a final concentration of 10 μ M.
- Within 10 minutes of substrate addition, measure donor emission (e.g. 450 nm) and acceptor emission (e.g. 610 nm) using a NanoBRET-compatible luminometer

Data analysis

- Generate NanoBRET ratios using the following formula: $\text{Acceptor}_{\text{sample}} / \text{Donor}_{\text{sample}}$
- Determine average NanoBRET ratio and SD for each set of data: 0%, 0.1%, 1%, 10%, and 100%.

- Subtract the average ratio for the 0% set from all other sets to obtain the corrected NanoBRET ratio, which represents the value for true energy transfer.
- Plot the corrected NanoBRET ratios and perform linear regression analysis in order to calculate the slope. The curve should be forced through $x, y = 0$ (since no BRET is possible in the absence of acceptor).
- Calculate the limit of quantitation (LOQ), which represents the minimum percentage of BRET pairs relative to the total donor population (fractional occupancy) that can be statistically distinguished from donor alone: $\text{LOQ} = (10 * \text{SD}_{0\% \text{ sample}}) / \text{slope}$.

Results/Conclusion

The LOQ for the SpectraMax i3x reader using the NanoBRET detection module was 0.58% fractional occupancy (Figure 1). Using the onboard luminescence detection mode, the LOQ was 2.6%. The higher sensitivity obtained with the NanoBRET detection module may benefit assays where signal is lower, while the onboard luminescence detection will be sufficient for many assays with stronger signal.

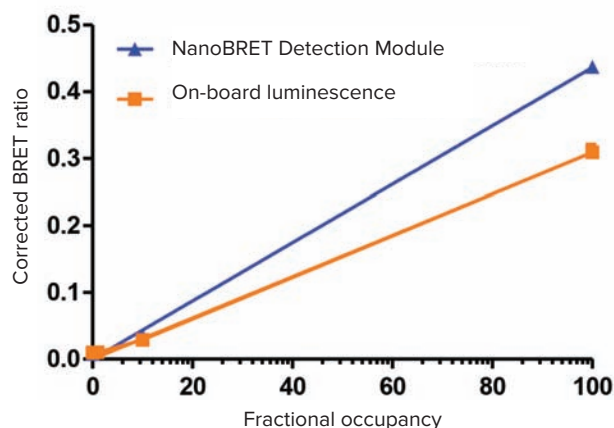


Figure 1. Results for SpectraMax i3x reader with NanoBRET Detection Module (blue) and on-board luminescence detection mode (orange). Curves were forced through $x, y = 0$. The calculated LOQ values were 0.58% for the detection module and 2.6% for the on-board optics (monochromators).

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Phone: +1.800.635.5577
 Web: www.moleculardevices.com
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USA and Canada +1.800.635.5577
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 China (Shanghai) +86.21.3372.1088
 Hong Kong +852.3971.3530

Japan +81.3.6362.9109
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