Sequential or simultaneous emission detection for BRET assays

Marjan Orban
BMG LABTECH, Ortenberg, Germany

- On-board (internal) reagent injectors for high throughput and fast kinetics
- A factor of 50 between positive and negative controls

Introduction

Bioluminescence Resonance Energy Transfer (BRET) is a system of choice for monitoring intermolecular interactions in vivo. BRET is an advanced, non-destructive, cell-based assay technology that is perfectly suited for proteomics applications, including receptor research and the mapping of signal transduction pathways. The assay is based on non-radiative energy transfer between fusion proteins containing Renilla luciferase (Rluc) and e.g. Yellow Fluorescent Protein (YFP). The BRET signal is generated by the oxidation of a coelenterazine derivative substrate.

For this application note the BRET2™ demo kit has been used to prove the feasibility of performing a BRET assay on a BMG LABTECH microplate reader. The BRET demo kit applies the cell-permeable and non-toxic coelenterazine derivative substrate DeepBlueC™ (DBC) and a mutant of the Green Fluorescent Protein (GFP2) as acceptor. These compounds show improved spectral resolution and sensitivity over earlier variants.

Materials & Methods

All materials were obtained through normal distribution channels from the manufacturers stated.

- BRET2™ demo kit, PE Life Sciences
- White 384-well OptiPlate™, PE Life Sciences

Experiments

A description for the development of BRET2™ protein-protein interaction assays is included with the demo kit. The following section focuses on the microplate reader settings recommended in the assay protocol.

BRET2™ demo kit reagents:
- Non-transfected CHO cell extracts
- Negative control [Rluc + GFP2 not fused together]
- Positive control [Rluc-GFP2 fused together]
- DeepBlueC™
- BRET2™ assay buffer

Assay Protocol (for a white 384-well plate)

1. Addition of BRET2™ assay buffer:
   A10-D12: 15 µL of BRET2™ buffer
2. Addition of 10 µL of each cell extracts (Fig.1):
   A10-A12: Non-transfected cells (blank)
   B10-B12: Neg. BRET2™ control [Rluc + GFP2]
   C10-C12: Pos. BRET2™ control [Rluc-GFP2]
   D10-D12: BRET2™ assay buffer

3. Automated injection of DBC and measurement:
   Insert the prepared plate in the instrument and fill the injector with DBC solution.
   A10-D12: Injection of 25 µL of DBC at 10 µM
   On-board reagent injectors allow the measurement of high throughput assays and fast kinetic signals. The data from the measurement was evaluated using the MARS Data analysis software.

Results & Discussion

When the donor and acceptor are in close proximity, the energy resulting from the catalytic degradation of the DBC is transferred from Rluc to GFP² which will then emit fluorescence at its characteristic wavelength.

The kinetic curves (raw data - blank) of the negative control are shown in Fig.2 for both channels. The low values of the 515 nm channel indicate that no resonance energy transfer occurred. Whereas the positive control shows reduced values at the 410 nm and elevated values at the 515 nm channel due to the BRET effect.
The calculated BRET ratio indicates the occurrence of protein-protein interaction in vivo. This type of detection eliminates data variability caused by fluctuations in light output which can be found with variations e.g. in assay volume, cell types, number of cells per well and/or signal decay across the plate.

In Fig. 3 the blank corrected BRET2™ ratios for both, negative and positive control, are shown and were determined as:

\[
\text{BRET2™ ratio} = \frac{\text{Em at } 515 \text{ nm} - \text{em at } 515 \text{ nm of non-transfected cells}}{\text{Em at } 410 \text{ nm} - \text{em at } 410 \text{ nm of non-transfected cells}}
\]

The MARS Data analysis software offers easy to use tools to do the ratio calculation in just 1 mouse click. The signal for negative and positive control for measurements using simultaneous dual emission reveals a value of around 0.06 and 3.3 respectively, which leads to a factor of around 50 and a clear discrimination between these controls. The signal for negative and positive control for measurements using sequential dual emission lead to values of around 0.05 and 1.9 respectively, which leads to a factor of around 40 (Fig. 4).

**Conclusion**

Ratiometrically quantifiable BRET2™ assays have been successfully measured using either sequential or simultaneous dual emission. However, the simultaneous dual emission option will lead to a higher assay window. Next to that, more data points per time can be monitored [50 measurements per second] and the measurement itself is a lot faster as two wavelengths are measured at the same time. The internal reagent injectors for 384-well plate format offer a great advantage for this kind of assay.