detect and identify

Application Note

Comparison of filter sets for BRET1 assays: ß-arrestin2 (ßARR2) recruitment to the vasopressin V2 receptor

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Introduction

G protein-coupled receptors (GPCRs) belong to a large family of transmembrane receptors and are targets for a large number of intercellular messengers such as hormones and transmitters. They are also called 7TM-Receptors referring to their structure of 7 transmembrane domains.

Upon agonist activation, most GPCRs recruit β-arrestins proteins. These multifunctional proteins have attracted particular interest because they desensitize G protein-dependent signal transduction, induce receptor endocytosis and promote β-arrestin-dependent signaling events.

When Rluc (*Renilla* Luciferase) is fused to the C-terminus of a 7TM receptor and YFP (Yellow Fluorescent Protein) is fused to the N-terminus of β-arrestin, the binding of β-arrestin to the receptor can be measured by BRET (Bioluminescence Resonance Energy Transfer).

This assay type holds many advantages over other cell based functional screening assays. One of these advantages is the fact that the assay can be used as a screening assay for virtually all 7TM receptors independent of their signaling pathway as the binding of β-arrestin to 7TM receptors is a universal process.

BRET1 Principle

Renilla Luciferase (Rluc) emits light at 480 nm when the substrate Coelenterazine is present. If a protein (e.g. a receptor) labeled with Renilla Luciferase (Rluc) binds to another molecule (e.g. β-arrestin) labeled with YFP, energy transfer will occur between Rluc and YFP when both proteins are brought into close proximity. The YFP molecule will then emit light at a higher wavelength (530 nm). Results are calculated as the ratio of YFP signal over Rluc signal.

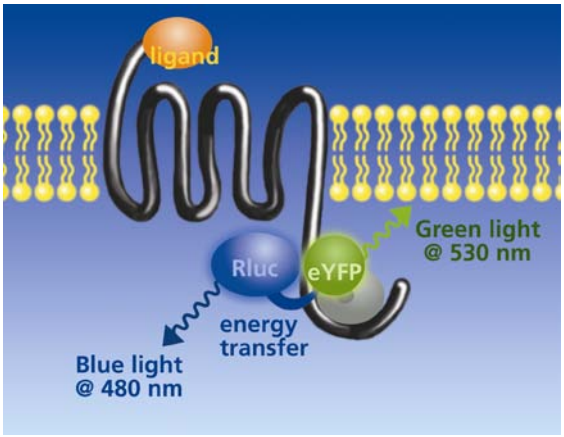

Figure 1: BRET principle

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Mithras

BRET and other colour luminescence applications are only possible with an extremely efficient optical system together with appropriate filters as used in the Mithras.

The Mithras LB 940 is a multimode plate reader with a unique optical design (DOPS – Dedicated Optical Path System) to ensure optimized performance for the detection technologies implemented. These are

- luminescence
- BRET/BRET²
- fluorescence
- FRET
- UV/VIS absorbance
- fluorescence polarization
- AlphaScreen®
- TRF
- HTRF®

In addition options like reagent injectors, temperature control and cooled PMT detection units are available.

Figure 2: Mithras LB 940 multimode reader

Filter Selection

Berthold Technologies has recently introduced high efficiency BRET and BRET² filter packages.

Here we compare the efficiency of the new optimized BRET filters to the classical BRET filters.

Assay Protocol

HEK293T cells were grown in complete medium (Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal bovine serum, 4.5 g/L glucose, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 1mM glutamine) (Invitrogen, CA). Transient transfections were performed using JetPEI (Polyplus Transfection, France), according to manufacturer's instructions.

HEK293T cells were seeded in 12-well plates and transiently transfected with 50 ng myc-V2R-Rluc or myc-V2R-Rluc8 and 500 ng of YFP-βARR2 or YPet-βARR2 expression plasmids. 24 h post-transfection, the cells were transferred into 96-well plates and left another 24 h before BRET measurements.

For BRET measurements, the cells were washed once with PBS (+Ca/Mg), and Coelenterazine H substrate (Interchim, France) was added at a final concentration of 5 µM and left for 10 min at RT. Cells were then stimulated by a saturating concentration (10^{-8}M) of Arginine vasopressin (AVP) (Calbiochem/Merck, UK) for 5 min.

BRET readings were performed using the Mithras multimode reader (Berthold Technologies) allowing sequential integration of luminescence with two filter settings (Rluc filter, 480/20nm or new 480/20nm with higher transmission; YFP/YPet filter, 530/25 nm or new 540/40nm). Emission signals at 530 or 540 nm were divided by emission signals at 480 nm, respectively (BRET ratio). The results are expressed in milliBRET units (mBU) corresponding to the BRET ratio multiplied by 1000.

Instrument Settings

Mithras LB 940 is operated through the Windows® PC software MikroWin 2000 which also serves as a data evaluation tool. With the standard configuration pre-set parameter files for BRET readings are supplied which can be modified according to individual needs. A

BRET assay involves two readings with the respective filters put in place.

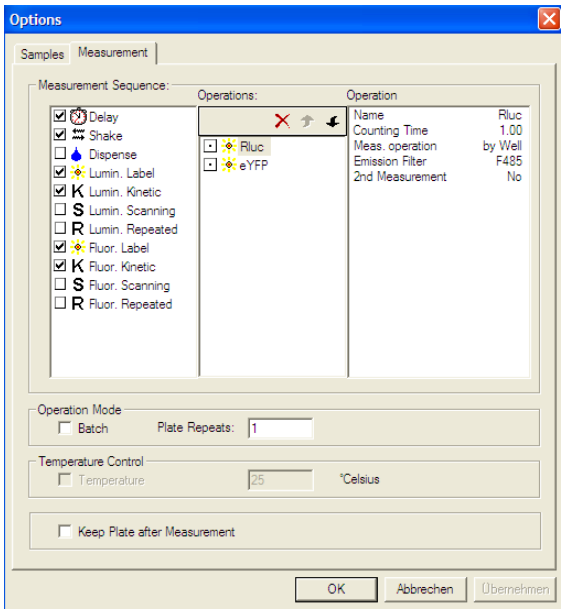

Figure 3: Operation sequence

Results

AVP-Induced βARR2-recruitment to the V2 receptor

The classical BRET donor Rluc and BRET acceptor YFP and the recently optimized Rluc8 and YPet variants were used (Kamal et al., 2009). The indicated vasopressin V2 receptor (V2R) donor and ß-arrestin2 (ßARR2) acceptor fusion constructs were coexpressed in HEK 293T cells and BRET values were determined in the absence and 5 min after the addition of the V2R agonist AVP using the classical (FilterSet1) and optimized filter (FilterSet2) settings. Whereas basal BRET values in the absence of AVP were negligible (2-10 mBU) (data not shown) and not statistically different between the two filter settings, a robust increase in BRET values was observed in the presence of AVP due to the recruitment of ßARR2 to the agonist-activated V2R.

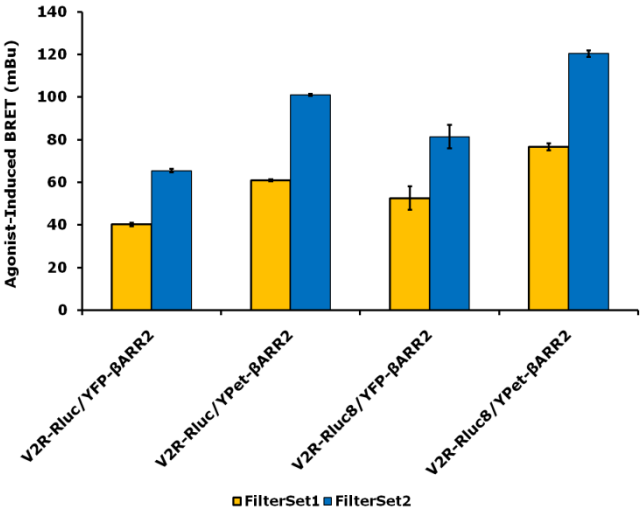

Figure 4: Agonist-induced BRET is shown for different BRET donor / acceptor pairs. Emission signals at 530 or 540 nm were divided by emission signals at 480 nm (BRET ratio). The results are expressed in milliBRET units (mBU) corresponding to the BRET ratio multiplied by 1000.

Conclusion

The results clearly show that the optimized filter settings provide significantly improved BRET signals for all donor/acceptor pairs. BRET values determined with the optimized filter settings were approximately 50% higher for each donor/acceptor combination tested.

Material

- HEK 293T cells
- Dulbecco's modified Eagle's medium (Invitrogen, CA)
- Fetal bovine serum (Invitrogen, CA)
- Penicillin (Invitrogen, CA)
- Streptomycin (Invitrogen, CA)
- Glutamine (Invitrogen, CA)
- JetPEI (Polyplus Transfection, France)
- 12-well plates (Corning, France)
- 96-well plates (PerkinElmer, France)
- PBS (Invitrogen, CA)
- Coelenterazine h (Interchim, France)
- Arginine vasopressin (AVP) (Calbiochem/Merck, UK)
- Mithras LB 940 multimode reader (Berthold Technologies)

- Filter set 1, classical
  (Berthold Technologies)
  480/20nm, (39450)
  530/25nm (39451)
- Filter set 2, optimized
  (Berthold Technologies)
  480/20nm (53425)
  540/40nm (53426)

Literature

Kamal M, Marquez M, Vauthier V, Leloire A, Froguel P, Jockers R and Couturier C: Improved donor/acceptor BRET couples for monitoring ß-arrestin recruitment to G protein-coupled receptors. *Biotechnol J,* 4:1337-1344 (2009)

Angers, S., Salahpour, A., Joly, E., Hilairet, S. et al., Detection of ß2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). *Proc. Natl. Acad. Sci. USA 2000*, 97, 3684–3689.