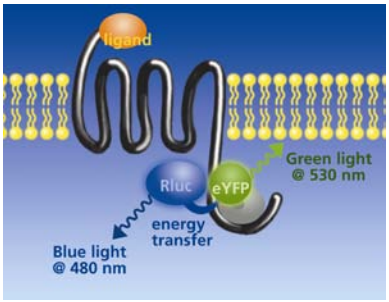
BRET (Bioluminescence Resonance Energy Transfer)

BRET is based on the fact that the energy derived from a luciferase reaction can be used to excite a fluorescent protein if the latter is in close proximity to the luciferase enzyme.

Especially in the field of G-protein coupled receptor research, BRET technology offers the opportunity to establish a homogeneous, universal and functional assay, taking advantage of the fact that ß-arrestin (which is naturally playing a role in the desensitation of the receptors) binds to the intracellular part of the activated receptor.

Advantages of BRET over other methods

- Non-radioactive and homogeneous
- Ratiometric signal minimises interferences from assay conditions
- No auto-fluorescence as no light source is required



Different BRET Methods

Over the last years different BRET methods have been developed. All of them have their limitations and benefits.

Various donor and acceptor pairs and their corresponding wavelengths can be found in the table below:

Method	Acceptor	Substrate	Acceptor Emission [nm]	Donor	Donor Emission [nm]
BRET 1	RLuc	Coelenterazine	480	eYFP	530
BRET 2	RLuc	Deep Blue C™ Coelenterazine 400a	395	GFP2	510
eBRET 2	RLuc8	Deep Blue C™ Coelenterazine 400a	395	GFP2	510
BRET 3	Firefly	Luciferin	565	DsRed	583
QD-BRET	RLuc/RLuc8	Coelenterazine	480	Qdot	605

The original BRET method using Coelenterazine as substrate is nowadays called BRET 1. It is characterized by strong signals and long life-time.

BRET 2 in comparison has better separated donor and acceptor emission peaks. This makes BRET 2 a better choice for screening assays where high signal to noise ratios are required. A clear limitation of BRET 2 is the low light emission and the short life-time.

Enhanced BRET 2 (eBRET) – leads to approximately 5-fold better signal as in the original BRET 2 version. eBRET uses a new Renilla luciferase mutant, Rluc8.

The Firefly luciferase in BRET 3 shows lower cellular autofluorescence at the emission wavelength (565 nm) but disadavantages are weak signals and overlap between donor and acceptor emission peaks.

A brand new BRET version is the Quantum Dot-BRET (QD-BRET). The emission peaks are clearly separated which makes QD-BRET ideal for screening applications. Disadvantages are the large size of the QD molecules (1.5 - 6nm) and the fact that genetical coding of QD-proteins is not possible. QD proteins cannot be expressed in living cells but must be added.

Literature: Bacart et al.(2008): The BRET technology and its application to screening assays, *Biotechnol. J.* 2008, 3, 311–324

BRET Filters

Due to low signals BRET and other colour luminescence applications need an extremely efficient optical system together with appropriate filters as present in the Mithras or TriStar.

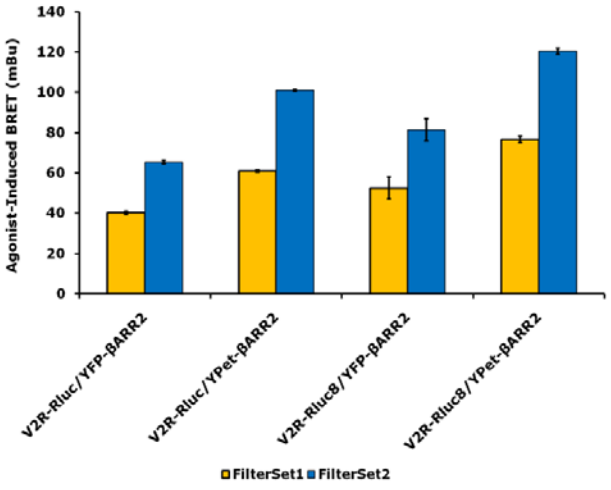
Berthold Technologies offers the following BRET filters. All filters have been tested extensively:

	Method	Wavelength [nm]	Order number	Wavelength [nm]	Order number
Standard filters	BRET 1	480/20	39450	530/25	39451
	BRET 2	400/70	39448	515/20	39449
High efficiency filters	BRET 1	480/20	53425	540/40	53426
	BRET 2	410/80	53427	515/40	53428

The four standard filters can be ordered as BRET package with order number 39350. Various Mithras models have the package already included.

The use of the high efficiency filters is recommended when either expression levels are low or when energy transfer has low efficiency.

BRET Filter comparison



Together with Ralf Jockers (Institute Cochin/INSERM, U567, Paris, France) the efficiency of the high efficiency BRET 1 filters versus the standard BRET 1 filters have been compared.

The classical BRET donor Rluc and BRET acceptor YFP and the new optimized Rluc8 and YPet variants were used (Kamal et al., 2009).

The results clearly show that the optimized filter settings provide significantly improved BRET signals for all donor/acceptor pairs. BRET values determined with the high efficiency filter selection were approximately 50% higher for each donor/acceptor combination tested (see detailed application note).

Berthold Technologies instruments:

Mithras LB 940 Multimode Reader
TriStar LB 941 Multimode Reader