

BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET²)

Principle

BRET is based on energy transfer from a bioluminescent donor (*Renilla* luciferase, Rluc) to a fluorescent acceptor protein (GFP). The BRET signal is measured by the amount of green light emitted by GFP as compared to the blue light emitted in luciferase reaction. The ratio of green to blue increases as the two proteins are brought into proximity.

Test

The bioluminescence energy transfer was evaluated on Plate CHAMELEON with a demo kit supplied by Packard. The demo kit contains CHO cell extracts expressing positive (GFP-Rluc(h) fused together) and negative (GFP + Rluc(h) not fused together) controls and DeepBlueC (luciferase substrate).

Filters 400 nm (bw 70)
 535 nm (bw 10)

Reagents BRET_{TM}² Demo Kit (Cat. No. 6310556, Packard BioScience)

Results

BRET signals (cps)

400 nm	1	2	Avg.
Non-transfected cells	28	46	53
pRluc(h) + pGFP	60526	72125	56053
pGFP-pRluc(h)	5793	5928	5244
Buffer	54	54	53
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535 nm	1	2	Avg.
Non-transfected cells	28	23	22
pRluc(h) + pGFP	192	217	190
pGFP-pRluc(h)	897	881	767
Buffer	28	24	37

RATIO (535 nm/400 nm)

	1	2	Avg.
pRluc(h) + pGFP (Neg.)	0.003	0.003	0.003
pGFP-pRluc(h) (Pos.)	0.151	0.146	0.148
Pos./Neg.	55.6	54.2	54.9

Conclusion

The GFP emission maximum is at 510 and therefore, the BRET signal obtained with 535 nm (bw 20) filter is somewhat lower than that of 510 nm (bw 30) suggested in the kit insert. Still, the the ratio of positive control to negative control is higher than specified in the insert (55 and 40, respectively).

The sensitive Plate CHAMELEON luminometer can read luminescence directly from the samples or through up to 3 filters. The BRET technology can be applied to Plate CHAMELEON providing a good platform for many assays in cell-based proteomics.

Product Information

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