Calcium (DAG and PKC) Biosensors

TECHNICAL NOTE

BioSens-All™

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The principal effector of the Gαq/11 pathway is phospholipase C-β (PLCβ), which catalyzes the cleavage of membrane-bound phosphatidylinositol 4, 5-biphosphate (PIP2) into the second messengers inositol (1,4,5) trisphosphate (IP3) and diacylglycerol (DAG). Binding of IP3 to its receptor on the endoplasmic reticulum (ER) results in calcium release. Increased concentrations of calcium and DAG lead to the activation of PKC. Consequently, biosensors monitoring DAG level and PKC activity have been developed in order to follow calcium flux following GPCR activation.

The DAG unimolecular biosensor is composed of C1b DAG-Binding Domains (DBDs) fused together via a flexible linker and double-tagged with *R*Luc and GFP. The PKC biosensor possesses a similar structure except that the C1b DBDs are replaced by PKC substrate domains, which interact together upon phosphorylation by PKC. Following GPCR activation, enrichment in DAG or substrate phosphorylation brings the GFP and *R*Luc in closer proximity resulting in a BRET signal increase (Figure 2).

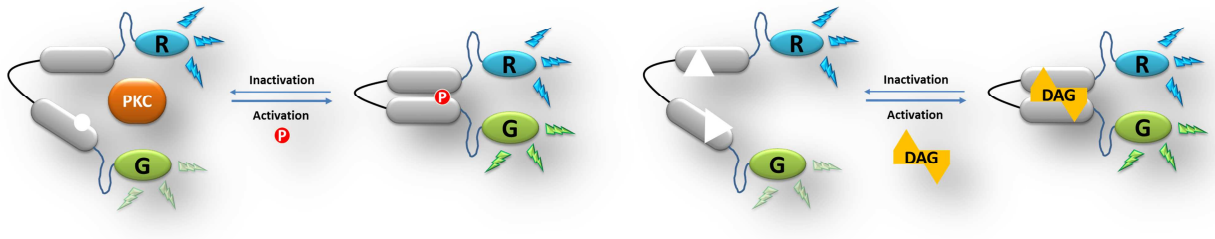
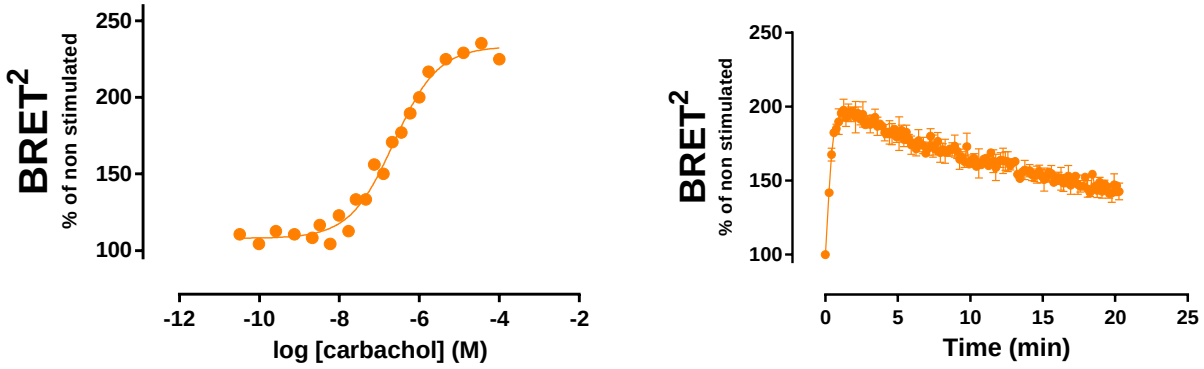


Figure 1. Structure of the BioSens-All™ DAG and PKC biosensors. R= *R*Luc and G= GFP

A



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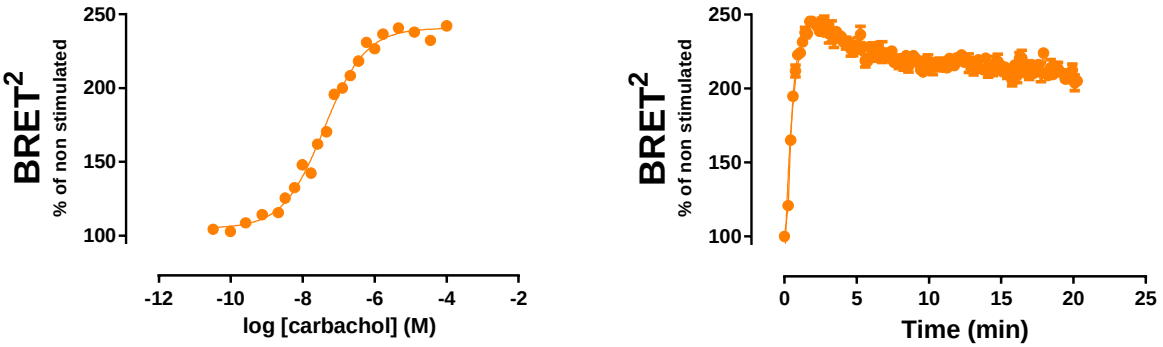

Figure 2. Indirect monitoring of calcium flux. A) Cells transiently overexpressing muscarinic M3 receptors and either the A) DAG biosensor or B) PKC biosensor were stimulated with increasing dose of carbachol. Ligand titration curves demonstrate a relative carbachol affinity of 233 nM and 39,9 nM when measured via DAG production or PKC substrate phosphorylation, respectively.