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**High-Throughput FRET Assays for cAMP Using the Epac1-based
Biosensor H188 Enable GPCR Agonist Drug Discovery**

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High-throughput screening of small molecule libraries is one means by which to identify new pharmacological agents that activate G protein-coupled receptors (GPCRs) to stimulate cAMP production so that ligands with beneficial medicinal properties are discoverable. Here we report a new microplate reader assay that allows high-throughput detection of cAMP with fast temporal resolution and wide dynamic range for cells treated with GPCR agonists. The assay uses conal HEK293-H188 cells that express the genetically encoded biosensor H188 in which the cAMP-binding protein Epac1 is flanked by mTurquoise2 Δ and ^{cp173}Venus-Venus FRET donor/acceptor fluorophores. Analysis of HEK293-H188 cell monolayers demonstrates that this assay is suitable to monitor the increase or decrease of cAMP levels that results when GLP-1, GIP, Glucagon, Adenosine-2B, TGR5, and NPY2R receptors are stimulated by their respective agonists. Accurate dose-response analysis is made possible by the nearly 10-fold greater Δ FRET that H188 exhibits when compared with the first generation biosensor Epac1-camps. By adapting this assay to studies of cyclic nucleotide analog action in living cells, we also define the properties of Epac1 activators and inhibitors with great precision. Thus, this microplate reader FRET assay enables GPCR drug discovery, while providing a new platform with which to advance cyclic nucleotide research.