High Sensitivity cAMP Assays in Primary Cells and Mesenchymal Stem Cells with the LANCE® *Ultra* cAMP kit.

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1 Abstract

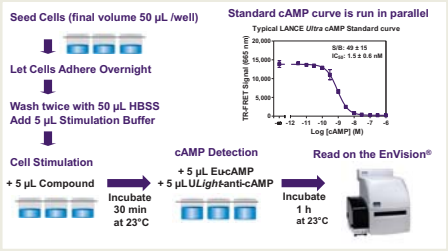
G-Protein coupled Receptors (GPCRs) remain the largest family of drug targets to date. One of the tools used during the development of new drugs to assess their activity at various $G_{\alpha s}$- and $G_{\alpha i}$-coupled GPCRs is the measurement of the modulation of intracellular cAMP concentration by these drugs. Recombinant cell lines stably expressing a receptor of interest are still widely used due to a number of advantages: their availability in large quantities, the possibility to examine a single receptor subtype, and the possibility to attain the high receptor expression levels that are needed for certain assays. However, receptor signaling and pharmacology can be modulated by several features that may not be well represented in such recombinant models, like the possibility for receptors to heterodimerize with other receptors belonging to the same family, to interact with other partner proteins, which may be absent from recombinant cells, and to trigger signal transduction in a cell-type-specific manner. For these reasons, the interest in primary and stem cells within research and drug discovery is increasing. As primary and stem cells are a precious material, more difficult to obtain and more valuable than recombinant cells, it is important to make the best use of them, and to have at one's disposal the most sensitive assays.

We have used here the new LANCE® *Ultra* cAMP kit to perform functional expression profiling of human Aortic Smooth Muscle Cells (AoSMC), human Mesenchymal Stem Cells (hMSC), Human Microvascular Endothelial Cells from the Lung (HMVEC-L) and Human umbilical Vein Endothelial cells (HUVEC).

2 LANCE *Ultra* cAMP Assay Principle

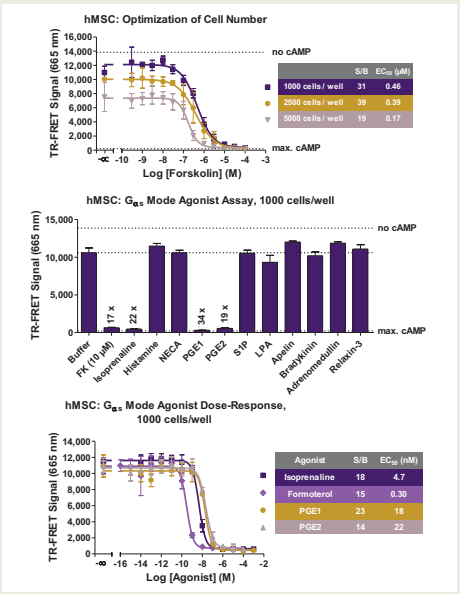
The LANCE® *Ultra* cAMP assay is a second-generation LANCE time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay designed to measure cAMP produced upon GPCR activation. The homogeneous two-component assay is based on the competition between europium chelate-labeled cAMP and cellular cAMP for binding to high-affinity anti-cAMP monoclonal antibodies labeled with the U*Light*™ dye.

3 Protocol (384-well Format)



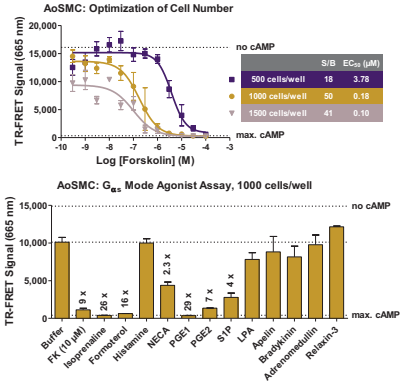
4 Human Mesenchymal Stem Cells (hMSC)

1000 hMSC cells/well were sufficient to get a very good assay window (S/B). The better response to formoterol, a ß$_2$-specific agonist, compared to isoprenaline, points to the presence of a ß$_2$-adrenergic receptor in hMSC. The response to PGE1 and PGE2 indicates the presence of some $G_{\alpha s}$-coupled prostanoid receptor (either DP_1, EP_2, EP_4 or IP_1).

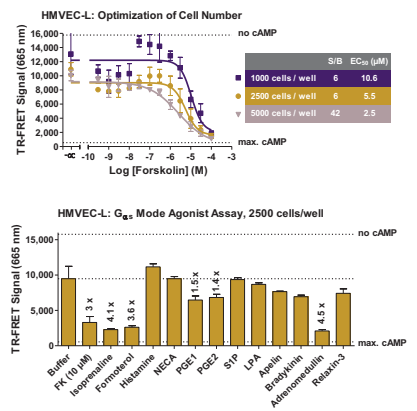


5 Human Aortic Smooth Muscle Cells (AoSMC)

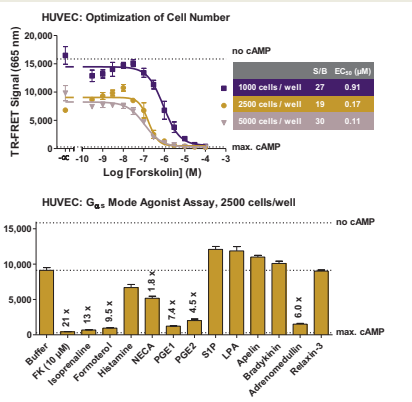
1000 AoSMC cells/well were sufficient to get a very good assay window (S/B). AoSMC response profile points to the presence of ß-adrenergic, prostanoid, adenosine and sphingosine-1-phosphate receptors in these cells.



6 Human Microvascular Endothelial Cells from the Lung (HMVEC-L)



7 Human Umbilical Vein Endothelial Cells (HUVEC)



8 Materials and Methods

Poietics® hMSC (Lonza # PT-2501), Clonetics® AoSMC (Lonza # CC-2571), Clonetics® HMVEC-L (Lonza # CC-2527) and Clonetics® HUVEC (Lonza # C2519A) cells were prepared and cultured according to Lonza's standard procedures. Cells were seeded in TC-treated white opaque bottom 384-well CulturPlates™ (PerkinElmer # 6007680), and left to adhere overnight. The next day, adherent cells were washed twice with HBSS and stimulated with 10 µM forskolin or the indicated agonists (10 µM Isoproterenol, 100 nM formoterol, 1 mM Histamine, 30 µM NECA, 10 µM Prostaglandin E1 (PGE1) or E2 (PGE2), 1 µM Sphingosine-1-phosphate (S1P), 5 µM Oleoyl-L-α-lysophosphatidic acid (LPA), 100 nM Apelin-13, 100 nM Bradykinin, 100 nM Adrenomedullin 1-52 (human), or 300 nM Relaxin-3) in stimulation buffer. cAMP was quantified with the PerkinElmer LANCE *Ultra* cAMP kit (TRF0262 /0263 /0264) according to the kit instructions.

9 Conclusions

- The LANCE *Ultra* cAMP assay detects GPCR activation in stem cells (hMSC) and in primary cells (AoSMC, HUVEC, HMVEC-L) in a 384-well format, using as few as 1000 or 2500 adherent cells/well without compromising assay performance.
- The screening of a small set of GPCR ligands demonstrated the presence of ß-adrenergic receptor(s) in the 4 cell types tested. The response of AoSMC, hMSC and HUVEC cells, and to a lesser extent of HMVEC-L cells, to PGE1 and PGE2 provides evidence of the presence of some prostanoid receptor in these cell types. Adrenomodulin activated HMVEC-L and HUVEC cells, but not AoSMC and hSMC cells.
- The S/B ratios obtained with the LANCE *Ultra* cAMP kit in these conditions enable an efficient use of these primary and stem cells for the pharmacological characterization of compounds.

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