BioTek
Booth #152

# Multiplexed GPCR Assays using HTRF® Technology

Laurence Jacquemart[1], Estelle NGarwate[1], Marie-Laure Lebreton[1], Peter Banks[2] and Xavier Amouretti[2]

1. Cisbio Bioassays, Drug Discovery, BP 84175, 30204 Bagnols-sur-Cèze Cedex, France
2. BioTek Instruments, Highland Park, P.O. Box 998, Winooski, VT USA 05404-0998

cisbio
Booth #138

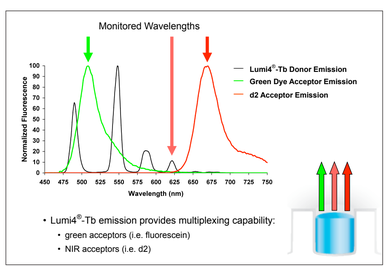
## Introduction

### Abstract

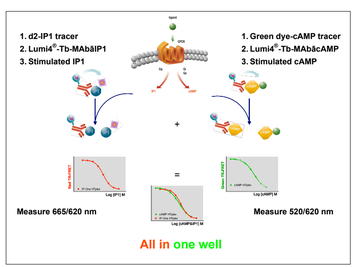
GPCRs carry information within cells via two major signaling pathways: regulation of cAMP levels and increases in intracellular $Ca2^+$ triggered by inositol (1,4,5) tri-phosphate (IP3). These signaling pathways are activated by the specific G protein associated with the receptor. Gs- and Gi-coupled receptors regulate cAMP levels while Gq-coupled GPCRs activate phospholipase C (PLC) and trigger the inositol phosphate (IP) cascade. Here we present an HTplex™ assay from Cisbio that allows for quantification of both cAMP levels and activation of the IP cascade in the form of IP1 using HTRF technology.

The IP1 assay is based on the competition between native IP1 produced by cells and IP1 tracer for limited amounts of a Lumi4®-Tb cryptate-labelled monoclonal antibody specific for IP1; the cAMP assays are similarly based on the competion between native cAMP produced by cells and cAMP tracer for limited amounts of a Lumi4®-Tb cryptate-labelled monoclonal antibody specific for cAMP. Differentiation between the two assays is based on using different acceptor dyes on the respective tracers: the cAMP tracer emits in the green portion of the electromagnetic spectrum while the IP1 tracers emit in the red. Data using a model system of stably-transfected vasopressin R2 in CHO cells will be presented.

### Introduction: Lumi4®-Tb Cryptate




### Multiplexing Assay Principle: Competitive Immunoassays from Cell Lysates




## Methods

### Reagents, Consumables and Instrumentation

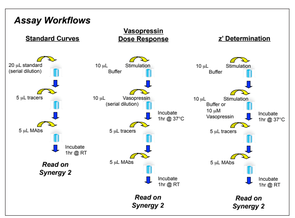
1. IP-one and cAMP reagent kits from Cisbio Bioassays, 2. CHO-Vasopressin R2 stably transfected
3. White 384-well microplates from Greiner, 4. Synergy™ 2 Multi-Mode Microplate Reader from BioTek Instruments

### Cells Preparation

CHO-V2R cells (stable transfection with the vasopressin-receptor subtype 2 gene) are cultivated in F12 medium. After counting the cells (3,000,000 cells/mL; viability: 96.3%), the culture media is diluted to obtain a concentration of 1,000,000 cells/mL. Then 30 µL are distributed in each well (30,000 cells per well). The plate is incubated at 37°C overnight. After that, the cell supernatant is aspirated and immediately replaced with 10 µL of stimulation buffer.

### Synergy 2 Instrument Settings

| | Read 1 | Read 2 | Read 3 |
|---|---|---|---|
| Emission filter | 620/10 | 665/8 | 520/10 |
| Excitation filter | 340/30 | | |
| Optics Position | Top 400 nm | | |
| Number of flashes | 10 | | |
| Lag time | 100 µs | | |
| Integration time | 300 µs | | |
| Sensitivity | Optimise on highest signal | | |
| Z' | Default | | |



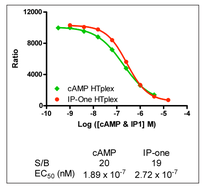

## Conclusions

- Both IP1 and cAMP levels quantified in the same well for Vasopressin R2 stimulation
- Simple "mix and read" format
- Kit sensitivity allows for quantification of pmol levels of IP1 and cAMP from 30,000 cells / well
- z' values for both IP1 and cAMP in excess of levels necessary for HTS campaigns
  — Could use less cells / well to reduce costs



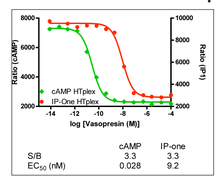
## Results

### cAMP and IP-One HT plex Standard Curves

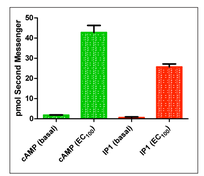



| | cAMP | IP-one |
|---|---|---|
| S/B | 20 | 19 |
| $EC_{50}$ (nM) | $1.89 \times 10^{-7}$ | $2.72 \times 10^{-7}$ |

### cAMP and IP-One HT plex CHO-VR2 30K cells/well



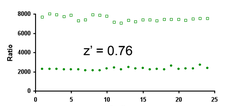

| | cAMP | IP-one |
|---|---|---|
| S/B | 3.3 | 3.3 |
| $EC_{50}$ (nM) | 0.028 | 9.2 |



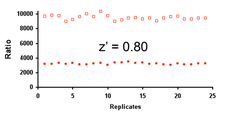

Note: Ratio signals converted to pmol second messenger using Prism curve fit to standard curves

### z' Determination

HTplex: cAMP




HTplex: IP-one




Note: $EC_{100}$ = 10 µM Vasopressin

### Emission Ratio Calculation[1]

**Ratio (red emission)** = (Signal at 665 nm / signal at 620 nm) x $10^4$

**Ratio (green emission)** = (Signal at 520 nm / signal at 620 nm) x $10^4$

1. The fluorescence ratio is a correction method developed by Cisbio International with an application limited to the use of HTRF® reagents and technology according to US patent 5,527,684 and its foreign equivalents Cisbio International has granted a license to BioTek Instruments.

$$z' = 1 - \left[ \frac{3\sigma_{basal} + 3\sigma_{EC_{100}}}{Ratio_{basal} - Ratio_{EC_{100}}} \right]$$

- Both cAMP and IP-one HTplex z' >>0.5