FlexStation® 3 System : Unwinding a GPCR pathway through Calcium flux and cAMP/IP3 production on a single instrument

Radhika Venkat,[1] Shengwen Zhang,[1] Helena Mancebo,[1] Simon Lydford[2] Amy DeGrandi[2] and Anita Kant[2]

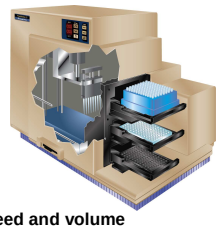
1: Multispan, Inc, 26219 Eden Landing Road, Hayward, CA 94545 2: MDS Analytical Technologies, 1311 Orleans Drive, Sunnyvale, CA 94089

Abstract

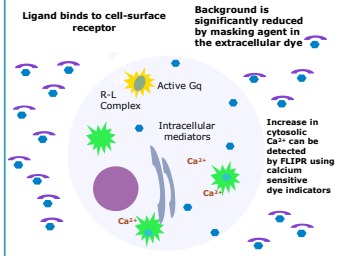
G protein-coupled receptors (GPCRs) constitute a major segment of drug discovery targets in the pharmaceutical industry. Accurate evaluation of drug candidates is critical during the screening process. Consolidating multiple readouts across the GPCR signal transduction pathways is a more comprehensive way of qualifying a drug candidate. Many commercial reagent platforms are available for measuring significant events downstream in the GPCR pathways, such as calcium flux, IP1 or cAMP production. These platforms require different detection modes and instrumentation. Here, we describe the use of The FlexStation®3 multimode reader with fluidics from MDS Analytical Technologies as a single instrument for capturing separate data sets in GPCR pathways. Multispan, Inc. has developed a large inventory of cell lines expressing specific GPCRs based on their unique expression technology platform and offers screening services. These GPCRs expressed in their native forms enable comprehensive investigations of effects of drug candidates on downstream signaling of each GPCR on the FlexStation®3 reader. Together, they provide a compact footprint for small to medium throughput screening and confirmation of drug candidates on multiple detection platforms. The data below present four GPCRs expressed in HEK293T cells evaluated with at least two detection platforms in each cell line.

FlexStation®3 Multimode Plate reader with fluidics:

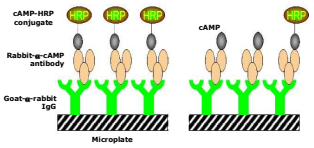
- Multichannel pipetter adds reagents from source plate to assay plate
- Simultaneous "Add and Read" feature
- Captures results in real time for fast kinetic assay
- 8-16 channel pipetter increases throughput compared to injector-based system where one well is assessed at a time
- No cross contamination of reagents
- No priming required
- Compound addition at desired time, height, speed and volume



A) MDS FLIPR® Calcium 4 kit for detection of calcium flux



B) MDS CatchPoint® cAMP competitive immunoassay



C) HTRF reagents from CisBio for detection of IP1 and cAMP:
cAMP or IP3 from the cells with activated GPCR compete with tracer molecules attached to acceptor dye for binding to donor Eu-cryptate or Tb-associated antibodies. The application runs on the TRF mode of FlexStation®3 system. The instrument is certified for HTRF assays after rigorous testing according to CisBio's guidelines.

Methods and Materials

Cell lines: CXCR4-HEK293T, GPR43-HEK293T, GPR120-HEK293T, CaSR-HEK293T

Reagents: FLIPR®-Calcium 4 kit (Molecular Devices R8142), Catch Point cAMP kit (Molecular Devices R8044), HTRF HiRange cAMP kit (CisBio 62AMCPEC), HTRF IP-one Tb kit (CisBio 621PAPEC). Compounds used as agonists were obtained from Sigma and R&D systems.

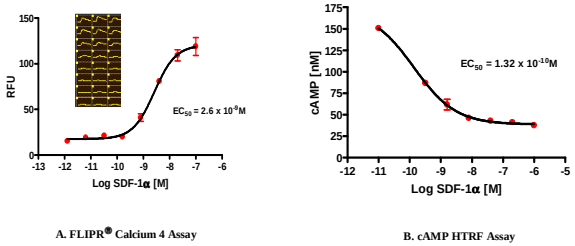
Calcium assay: Cells seeded and grown overnight in poly-D-lysine coated plates were incubated for one hour with the assay dye solution. Calcium flux was monitored upon addition of compounds in the FlexStation®3 system according to the manufacture's protocol.

cAMP assay: Cells seeded and grown overnight in poly-D-lysine coated 384 or 96 well plates were incubated with respective agonist for 15 min followed by incubation with forskolin at EC_{80} concentration. Inhibition of forskolin stimulated cAMP was monitored using CisBio HiRange kit or Molecular Devices CatchPoint® kit.

IP-One Tb assay: Cells seeded and grown overnight in poly-D-lysine coated 384 or 96 well plates were incubated with agonist for 1.5-2 hours. Dose-dependent accumulation of IP1 was monitored using IP-One Tb kit.

Results

1) Calcium flux and cAMP assays for chemokine receptor CXCR4

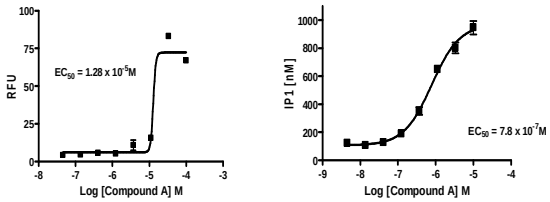


A. FLIPR® Calcium 4 Assay

B. cAMP HTRF Assay

Figure 1. A. Dose dependent intracellular Ca^{++} flux assay upon treatment with agonist in HEK293T cells stably expressing human CXCR4 receptor. Cells were transiently transfected with a chimeric G protein Gq_{i5}. B. Inhibition of forskolin stimulated cAMP upon treatment with ligand using CisBio HiRange cAMP kit (62AMCPEC).

2) Calcium flux and IP-One Tb assays for calcium sensing receptor (CAS)

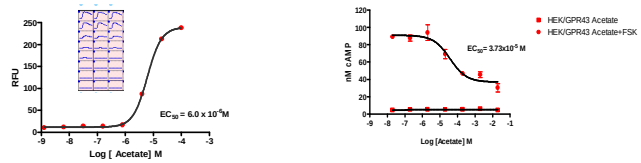


A. FLIPR Calcium 4 assay

B. IP-One Tb Assay

Figure 2. Compound A, an agonist for calcium sensing receptor (CASR) shows difference in response with Ca^{++} flux and IP1 assay. A. Dose-dependent Ca^{++} flux upon treatment with compound in HEK293T cells transiently expressing human CAS receptor. B. Dose-dependent IP1 accumulation upon treatment with compound using CisBio IP-One Tb kit (621PAPEC). There is a significant shift in the EC_{50} values with two assays.

3) Calcium flux and cAMP assays for free fatty acid receptor GPR43

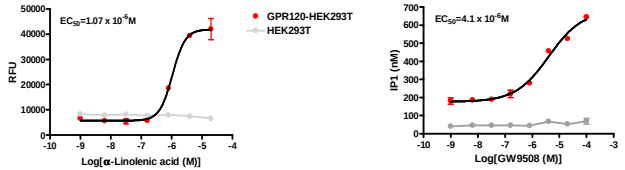


A. FLIPR Calcium 4 Assay

B. Catch Point cAMP Assay

Figure 3. A. Dose-dependent Ca^{++} flux assay upon treatment with agonist in HEK293T cells stably expressing human GPR43 receptor. B. Dose-dependent inhibition of forskolin-stimulated cAMP accumulation upon treatment with ligand using MDS CatchPoint® cAMP kit (R8044).

4) Calcium flux and cAMP assays for free fatty acid receptor GPR120



A. FLIPR Calcium 4 assay

B. IP-One Tb Assay

Figure 4. A. Dose-dependent Ca^{++} flux upon treatment with agonist in HEK293T cells stably expressing human GPR120 receptor. B. Dose-dependent IP1 accumulation upon treatment with agonist using CisBio IP-one Tb kit (621PAPEC).

Conclusion:

1) Monitoring GPCR pathway at multiple points ensures efficient screening of drug candidates.

2) Calcium flux and generation of IP1 or cAMP as downstream products are important readouts during drug screening process.

3) Slow binding compounds missed by calcium flux can be detected using IP-one Tb assay.

4) Promiscuous G-proteins can facilitate calcium flux assays of receptors that are not naturally coupled to $G\alpha_{q/11}$.

5) The FlexStation®3 multimode reader with fluidics provides a single instrument platform to perform multiple detection assays for individual GPCRs.

MULTISPAN
One-Stop Shop for GPCR Research

MDS Analytical Technologies
Science advancing health