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Division-Arrested Cells Expressing G Protein-Coupled Receptors

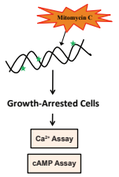
Radhika Venkat, Jose Valle, Patricia Yeung
Helena Mancebo and Shengwen Zhang

Multispan, Inc.
26219 Eden Landing Road , Hayward , CA 94545-3718
Correspondence: Shengwen.zhang @ multispaninc.com

ABSTRACT

Cell-based functional assays for high-throughput screening (HTS) have become widely adopted by the pharmaceutical industry. High cost of cell culture, labor-intensive nature of the assays and inherent variability in the cellular responses are among the hurdles for extensive applications of cell-based assays in HTS. The consistent performance of the cells in an assay can at times be greatly affected by changes in target expression as a result of very high cell passages. A well characterized bank of cells can be division-arrested and cryopreserved, and these division-arrested cells can substitute dividing cells and provide consistency in cell-based assays. Multispan has optimized the assay conditions of division-arrested cells for a panel of G protein-coupled receptors (GPCRs). The pharmacological performances of GPCRs in these cells are demonstrated by consistent assay windows and EC_{50} values. Multispan has been using these cells for identifying new drug targets and discovering therapeutic interventions in high-throughput screening and compound profiling applications.

INTRODUCTION



Advances in high-throughput detection technologies have resulted in an increased use of cell-based functional assays in early drug discovery, in particular for the G protein-coupled receptors (GPCRs). GPCRs are involved in many physiological functions, and have been implicated in various diseases and pathological states. These receptors are targets of approximately 30% of all medicinal drugs. Stimulation of the receptors by extracellular ligands triggers signaling cascades mainly through activation of Gαq/11, Gαi/o and/or Gαs of the heterotrimeric G protein family. Multispan has developed high-throughput assays assessing the activation of these pathways in mammalian cells expressing a large panel of GPCRs. Taking advantage of the convenience and consistency of division-arrested cells, Multispan has optimized the conditions for Ca^{2+} mobilization and cAMP assays in mitomycin C-treated cells. In this report, we present data generated from division-arrested cells that can be used for high-throughput screening and compound profiling applications.

MATERIALS AND METHODS

Cells: CHO-K1, HEK293T or RH7777 cells transiently or stably expressing GPCRs.

Division arrest: Cells seeded and grown overnight were incubated for two hours with mitomycin C (Sigma,M4287). The cells were frozen at -80°C overnight and transferred to liquid nitrogen for long-term storage.

Compounds: Compounds were purchased from R&D systems and Sigma.

Calcium Mobilization Assay: Cells cultured overnight in Poly-D-Lysine-coated plates were incubated for one hour with buffer containing calcium indicator dye FLIPR® Calcium 4 (Molecular Devices, R8142). Calcium flux was monitored upon addition of compounds using FlexStation III (Molecular Devices).

cAMP Assay: Cells treated with compounds were subjected to cAMP assay using cAMP Hi-Range Kit (Cisbio, 62AM6PEC).

Data analysis: Data were analyzed using Prism 4.03 (GraphPad).

Ca^{2+} Assays in Cells Expressing mGlu6 Receptor

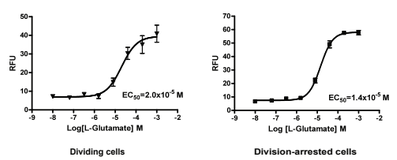


Figure 1. Dose-dependent stimulation of Ca^{2+} flux in HEK293T cells stably expressing the human metabotropic glutamate receptor mGlu6. The agonist elicited signals with comparable efficacy and potency in both dividing and division-arrested cells.

Ca^{2+} Assays in Cells Expressing 5-HT2A or 5-HT2C Receptor

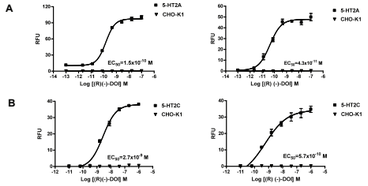


Figure 2. Dose-dependent stimulation of Ca2+ flux by DOI in CHO-K1 cells stably expressing the human serotonin receptors 5-HT2A (A) or 5-HT2C (B). Division-arrested cells offer similar signal/noise ratios and assay sensitivity to dividing cells.

Ca^{2+} Assays in Cells Expressing LPA3 or GPR40 Receptor

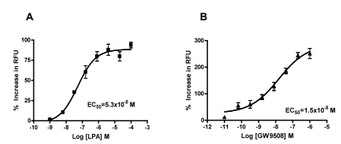


Figure 3. Dose-dependent stimulation of Ca^{2+} flux was monitored upon treatment with agonists. A) Division-arrested RH7777 cells expressing the human lysophospholipid receptor LPA3. B) Division-arrested CHO-K1 cells stably expressing the human free fatty acid receptor GPR40. The signal windows and EC_{50} values are comparable to those acquired using dividing cells with better consistency over an extended period of time.

Cyclic AMP Assays in Cells Expressing CCR5 or CB1 Receptor

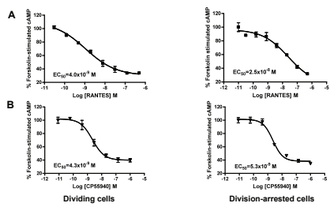


Figure 4. Inhibition of forskolin-stimulated cAMP levels by agonists in HEK293T/CHO-K1 cells stably expressing the human chemokine receptor CCR5 (A) or the human cannabinoid receptor CB1 (B). Both dividing and division-arrested cells responded to agonists with similar pharmacological properties.

Cyclic AMP and Ca^{2+} Assays in Cells Expressing DP2 Receptor

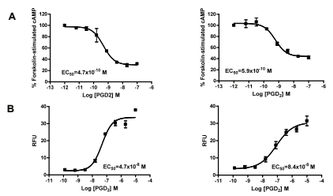


Figure 5. Inhibition of forskolin-stimulated cAMP levels (A) and stimulation of Ca^{2+} flux (B) by PGD_2 in HEK293T cells stably expressing the human prostanoid DP2 receptor. Both cAMP and Ca^{2+} assays revealed comparable signal windows and EC_{50} values in dividing and division-arrested cells.

Ca^{2+} Assays in Cells Transiently Expressing TRH Receptor

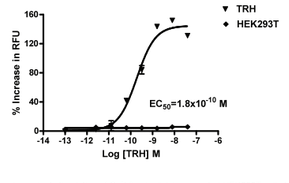


Figure 6. Dose-dependent stimulation of Ca^{2+} flux in division-arrested HEK293T cells transiently expressing the human thyrotropin releasing hormone (TRH) receptor. The signal windows and EC_{50} values are comparable to those acquired using dividing cells with better consistency than transient assays in different batches (data not shown).

SUMMARY

- Division-arrested cells offer convenient tools for cell-based high-throughput screening.
- Comparable EC_{50} values have been achieved from division-arrested and dividing cells, demonstrating excellent pharmacological performance of the receptors used.
- Division-arrested cells can be successfully prepared for different assay formats from various parental cells expressing GPCRs stably or transiently.
- Multispan offers division-arrested cells for compound screening, profiling and other applications.