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Developing Cell-Based Functional Assays for Difficult-to-Express GPCRs

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Abstract

G-Protein-Coupled Receptors (GPCRs) are the most validated class of "druggable" targets due to their access to important pathways and their success in the marketplace. Because of the structural and functional commonalities among this large family of receptor proteins, it is critical and challenging to deconvolute the specificity of small molecule and antibody drug leads. A comprehensive panel of functional assays for 125 GPCRs have been developed by Multispan to address this need, including Free-Fatty-Acid (FFA) receptor family and glutamate (mGlu) receptor family, important drug targets for obesity/diabetes and psychiatric/physiological disorders.

Assay development for FFA and mGlu families of GPCRs have been stumbling blocks in drug discovery. Multispan has addressed this outstanding issue by leveraging robust proprietary GPCR expression technologies and assay development expertise. In this report we demonstrate success in developing assays for the entire FFA receptor family and for selected members of the mGluR family.

We present data using a stable cell line based assay for GPR40 (FFA1) to determine if a particular compound is specific to this target of interest. Forty compounds were tested for agonist activity to GPR40 in a validated Ca^{++} assay with consistent performance confirmed by EC_{50} data with a known ligand.

Free Fatty Acid (FFA) Family of GPCRs

Ligands for recently de-orphanized FFA receptor family:

FFA Receptor Target	Ligand
GPR40	medium and long chain FFAs
GPR41	short-chain carboxylic acids
GPR43	short-chain carboxylic acids
GPR120	long chain FFAs

GPR41 shares 98% of amino acid homology with GPR42 and is closely related to GPR43. Functional roles of all these receptors are still under discussion and the expression patterns among the family members are different.

Human GPR40 in Ca^{++} Assay

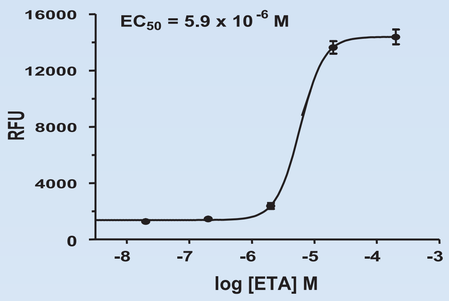

Figure 1. Stable GPR40-HEK293T cells were transiently transfected with a $G_{\alpha qi5}$ expression vector and the intracellular Ca^{++} dose response was monitored upon treatment with ligand ETA.

GPR41 in Ca^{++} and cAMP Assays

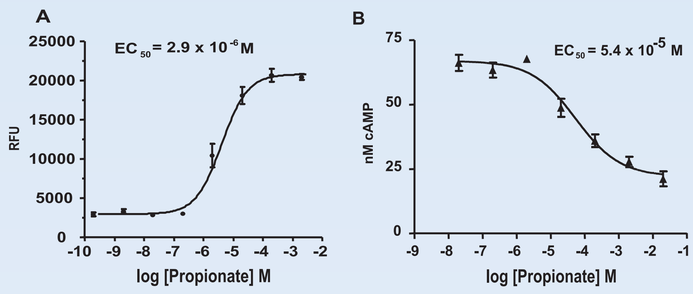

Figure 2. A. Stable GPR41-HEK293T cells were transiently transfected with a $G_{\alpha qi5}$ expression vector and the intracellular Ca^{++} dose response was monitored with Ca^{++}4 Kit from Molecular Devices upon treatment with ligand. **B.** Stable GPR41-HEK293T cells were preincubated with Sodium Propionate for 10 minutes, followed by incubation with Forskolin at 10 μM for 15 minutes. Cells were lysed and tested by cAMP kit from Molecular Devices.

Characterization of GPR43-HEx™ Cell Line

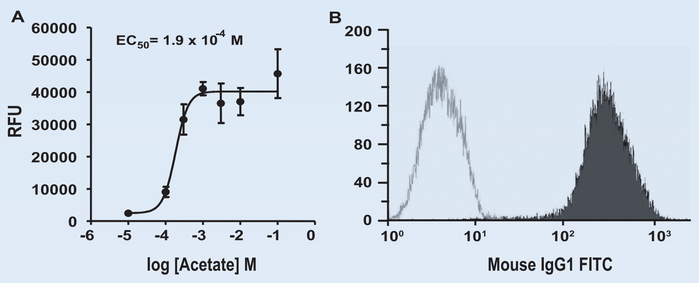

Figure 3.A. Dose response curve of intracellular Ca^{++} in the proprietary stable GPR43-HEx™ cell line. **B.** FACS analysis of GPR43 surface expression in the stable GPR43-HEx™ cells using anti-FLAG and FITC conjugated anti-mouse antibodies.

Newly-Discovered FFA Receptor GPR120

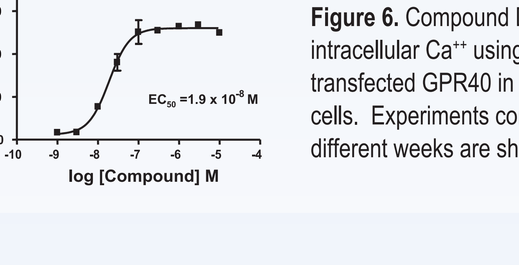

Figure 4. HEK cells were transiently transfected with GPR120 and promiscuous protein $G_{\alpha 16}$. The intracellular Ca^{++} dose response was monitored upon treatment with the ligand.

Compound Profiling

Receptor profiling helps to predict off-target effects early in development, saving drug development time and cost.

The functional data yield higher value results. Multispan offers the choice of using transient transfection to avoid clonal effect or stable clones with optimal S/N and EC_{50}. Researchers can choose between Multispan's full panel of 125 pre-validated cell-based assays or use our library of 320 full length GPCR cDNAs cloned in Multispan's proprietary vector to create a custom panel.

Quick turn-around time: 2 weeks for validated assays.

40 Compounds Tested for GPR40 Agonist Activity in Ca^{++} Assay

	Compound: 10 μM				Compound: 1 μM			
	hGPR40-HEK293T		HEK293T Control		hGPR40-HEK293T		HEK293T Control	
Compound #	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
1	1386	167	2606	469	2356	274	2174	163
2	2809	254	2365	304	2426	213	1885	364
3	5552	1749	2466	110	2263	446	1687	376
4	5825	315	2121	490	1422	186	1846	610
Positive 1	11297	1670	1757	116	10858	340	1424	88
Positive 2	11417	597	1211	201	10124	563	756	78
Positive 3	9860	945	1724	352	10254	636	1251	315
Negative	2988	380	2175	310	2330	294	1672	385

Figure 5. GPR40-HEK293T cells (S/B>10) were used to profile 40 compounds for the agonist activity targeted against FFA1 receptor.

Consistency Between Weekly Experiments

Figure 6. Compound DRC for intracellular Ca^{++} using transiently transfected GPR40 in HEK293T cells. Experiments conducted in 3 different weeks are shown.

mGlu Family of Receptors

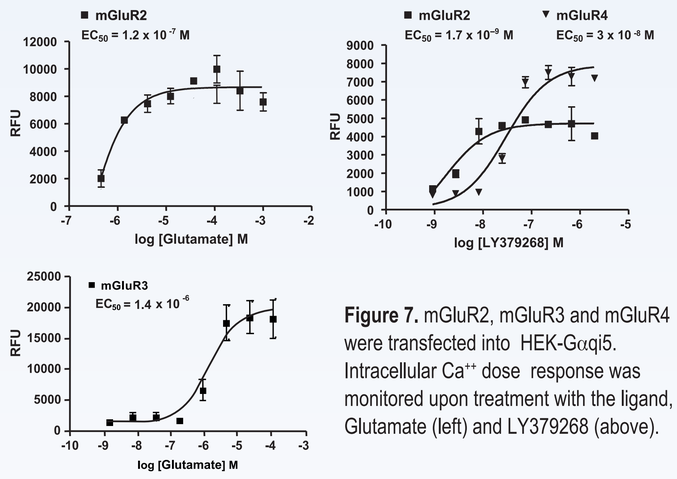

Figure 7. mGluR2, mGluR3 and mGluR4 were transfected into HEK-Gαqi5. Intracellular Ca^{++} dose response was monitored upon treatment with the ligand, Glutamate (left) and LY379268 (above).

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