G-Protein Coupled Receptors: A Fast, Convenient Method to Identify Inhibitors of Ligand Binding and Second Messenger Accumulation

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Abstract

We have developed a robust, convenient platform for HTS assays involving G-Protein Coupled Receptors (GPCRs). The assays are conducted with Meso Scale Discovery's Multi-Array™ technology, which combines array technologies and electrochemiluminescence detection. This poster presents two classes of assays involving GPCRs. The first assay measures receptor-ligand binding directly and uses only 0.15µg membrane per well in 384-well plates. Cellular membranes containing the melanocortin receptor (subtype 5) are immobilized on MSD High Bind Multi-Array plates. The corresponding ligand, NDP-α-MSH, is labeled with MSD's electrochemiluminescent label (Ruthenium (II) tris-bipyridine N-hydroxysuccinimide, "TAG"), for use in this assay. The format is compatible with screening workflows with excellent signal to noise ratios. The second assay is a functional assay for GPCR activity based on detection of cAMP. In this assay, stimulation with the ligand (NDP-α-MSH) generates cAMP in-situ, which is quantified using a competitive immunoassay. Both assays are fully automated, read times are fast (<90 seconds per plate) and Z' scores are above 0.6.

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❷ Meso Scale Discovery Multi-Array™ Technology

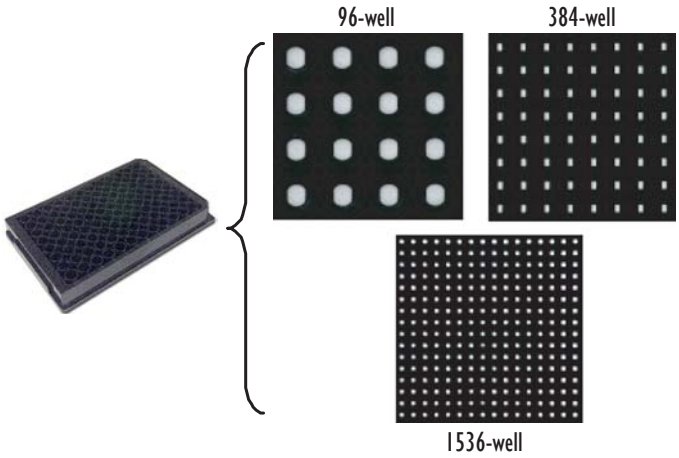
Sector HTS™ Reader Features

- Highly sensitive, ultra high-throughput
- Designed for high-throughput screening (HTS) and automated assay development
- Custom optics with telecentric lens design and CCD imaging detection
- High-speed motion control systems
- Electrochemiluminescence (ECL) detection

Plate Features

- Disposable Plates
- Carbon Electrodes with high binding capacity
- Suitable electrochemistry for ECL
- Biocompatible: direct immobilization of avidin, IgG, membrane fragments, intact cells, etc.
- Functional Assays: simple binding reactions, GPCRs, enzyme cascades, post-translational modification, etc.

Sector Images



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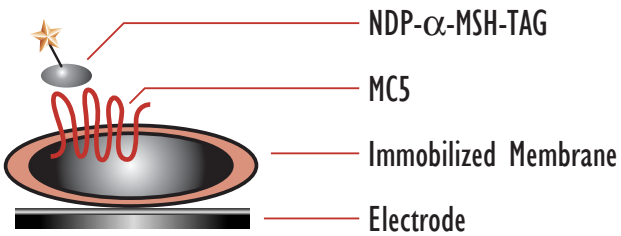
❸ Simple, No-Wash Protocol with Immobilized GPCR Membranes



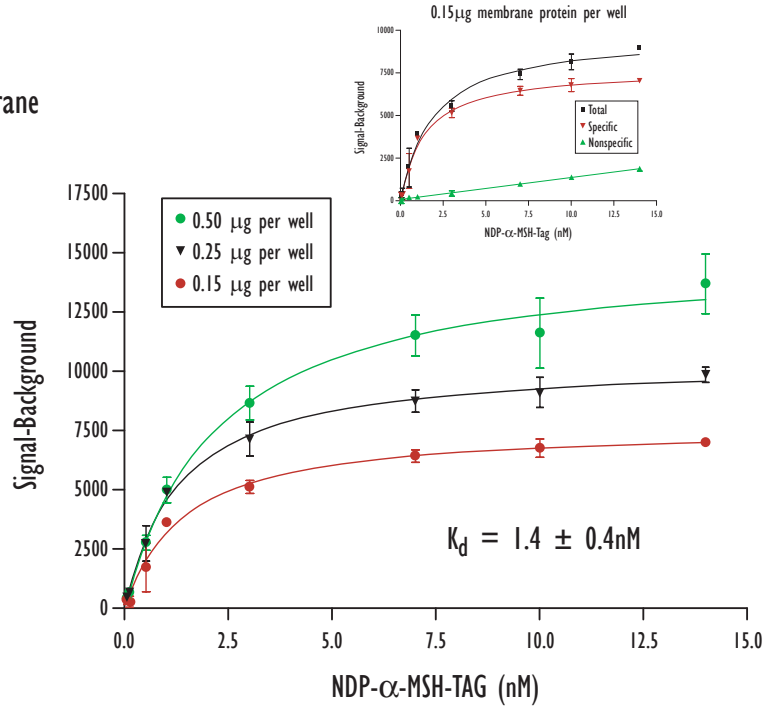
Membranes Incubated on MSD proprietary coated 384-well High Bind Multi-Array plates,1h

↓

Addition of labeled ligand, 1h

↓

Plate Imaged on MSD's Sector HTS reader



GPCR binding activity is preserved in the immobilized membranes. NDP-α-MSH-TAG binding to Melanocortin 5 (MC5) was detected using as little as 0.15µg membrane protein per well (13pmol/mg), S/B=18. Inset: Signal observed with MC5-containing membranes is compared to the nonspecific signal observed with parental HEK293 membranes that do not express MC5.

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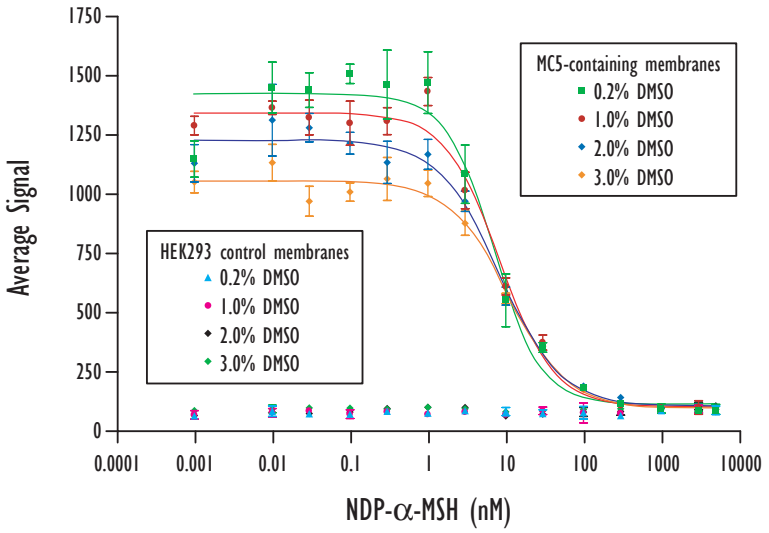
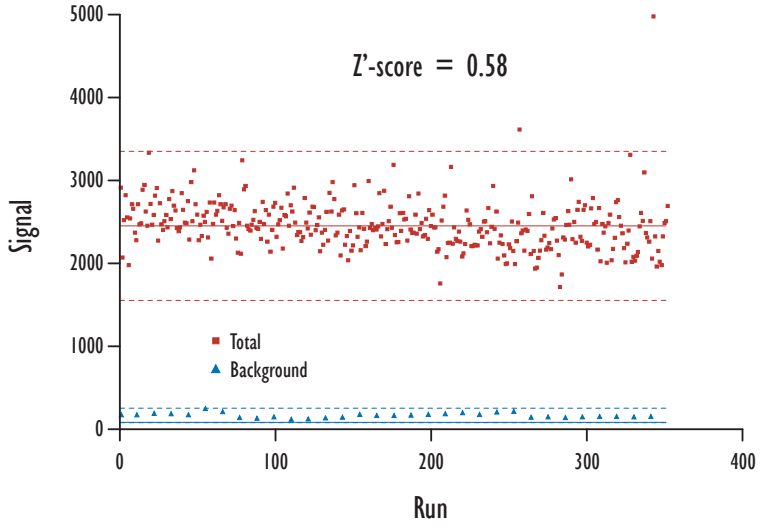
❹ DMSO Tolerance Studies: Binding Characteristics Maintained for Screening Compound Libraries

Membranes were dispensed at 0.15µg per well in MSD proprietary coated 384-well High Bind Multi-Array plates. The binding of labeled NDP-α-MSH to MC5 in the membranes was measured in the presence of increasing amounts of unlabeled ligand. The competition study was conducted with 0.2-3.0% DMSO present during the binding reactions, demonstrating the tolerance of the assay format to DMSO.



❺ Assay is Compatible with Screening Workflows

This non-washed assay (total time 2h) was conducted with automated liquid handling and read on an MSD Sector HTS reader (<1min/plate). Total signal is that produced from MC5 receptor-expressing cell membranes challenged with 1nM NDP-α-MSH-TAG, while background is the signal obtained for the same samples in the presence of a 200-fold excess of unlabeled NDP-α-MSH.



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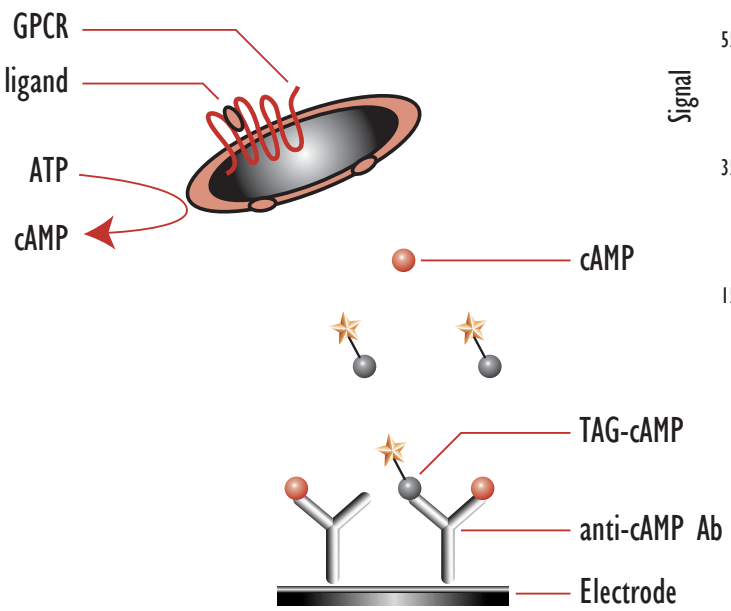
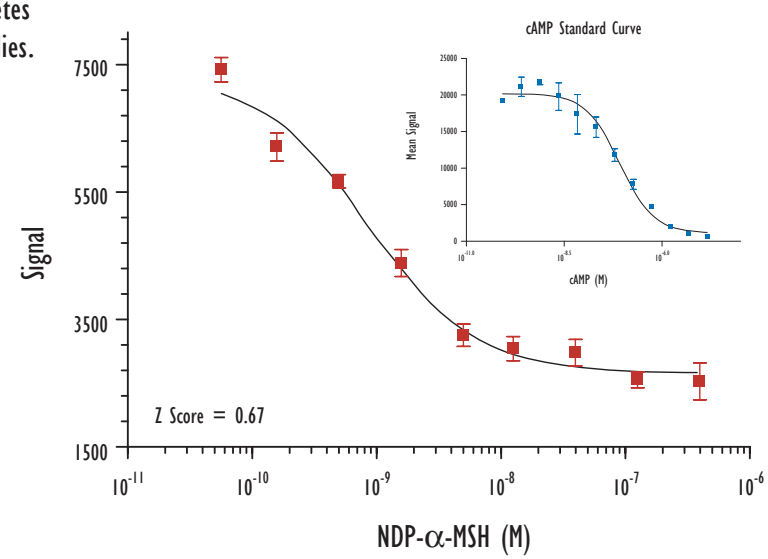
6 Functional Assay for GPCR Activity Based on Detection of cAMP in MC5 Membranes

This assay is a non-washed, completely automated, 384-well format.

cAMP produced by stimulation of MC5 with NDP-α-MSH competes with labeled cAMP for binding to immobilized α-cAMP antibodies. The EC50 of the ligand is in agreement with published data.



Stimulated cAMP in MC5 Membranes



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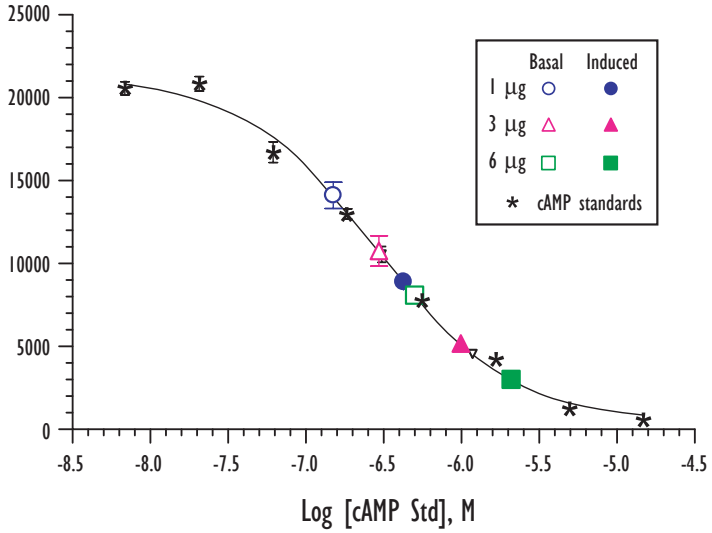
7 Titration of MC5-Containing Membranes Demonstrates Agonist Potency for cAMP

The basal and stimulated cAMP levels generated from MC5 membranes remain in the linear range of the standard curve when using as little as 1 – 6 µg of membranes. The cAMP levels from membranes in the presence (induced) or absence (basal) of NDP-α-MSH are shown along the standard curve.



8 Conclusions

1. We have developed a simple, robust GPCR assay that detects MC5 receptor binding to labeled NDP-α-MSH in a non-washed format using only 0.15µg total membrane protein per well.

2. Receptor-ligand binding characteristics were insensitive to the addition of DMSO, demonstrating compatibility of the assay format with screening compound libraries.

3. Both the receptor-ligand and functional GPCR assays are run in 384-well plates in a completely automated format with fast read times and excellent Z'-scores.

3. Agonist potencies were confirmed in a competitive cAMP reporter assay using MC5 membranes. NDP-α-MSH induced cAMP production was shown with as little as 1–6 µg of membrane per well.

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