MSD[®] GPCR-Ligand Binding Assay Demonstration Kit: Melanocortin 5 MULTI-ARRAY[™] 384 Plate

I. Materials Included

		Storage
MSD Catalog Items		
	MSD Read Buffer T, surfactant free (4x)	RT
	MSD Blocker A	4°C
R&D Custom Items		
	MULTI-ARRAY PEI 384 Plate (High Bind)	RT
	MC5 receptor-containing cell membranes	- 80°C
	Control membranes	- 80°C
	MC5 Binding Buffer (10X)	4°C
	MSD TAG™ labeled NDP-α-MSH	4°C
	NDP-α-MSH (unlabeled)	4°C

II. Other Materials & Equipment (not supplied)

- Deionized water for diluting Binding Buffer and Read Buffer.
- □ 37°C water bath or incubator for quick thawing membranes.
- □ Automated or manual multi-channel liquid handling equipment. Must accurately deliver $2 \mu L$ to a precise location in X, Y, Z coordinates within each well of a 384-well plate.
- □ Hand pipettes, tubes, source plates, tips, etc. for making serial dilutions of ligand. Required sizes will depend upon the scale of experiment.
- □ Syringe (1 or 3 mL, depending upon size of experiment) with a 26 gauge, 5/8 inch needle.
- □ Adhesive plate seals and/or cover plates.



III. Preparation & General Notes:

Prepare working stock of MC5 Binding Buffer:

- 1. Approximately 20 mL is required per 384-well plate.
- 2. Dilute 10X MC5 Binding Buffer to 1X with deionized water.

Prepare membranes:

- 1. Determine the number of wells to be used in the experiment. Each will receive 2 μ L (0.3 μ g) of diluted MC5 receptorcontaining or control membranes. Remember to include extra for "dead" volume in the pipetting technique when performing calculations for membrane preparations. A 10-12 μ L cushion per well is recommended when using automation with a source plate, or a 10-15% excess volume per well when hand-spotting membranes.
- 2. Quick-thaw membranes in a 37°C water bath for one minute.
- 3. Collect aliquots to one tube (if using multiple stock tubes) and process 6 times using a syringe with a 26G 5/8 needle, keeping the flat end of the needle against the sidewall of the tube.
- Dilute membranes from the stock concentration of 1.5 μg/μL to 0.15 μg/μL (1:10) in cold 1X MC5 Binding Buffer.
- 5. Keep on ice until ready to dispense.

Prepare unlabeled ligand for competition curve (if desired):

- 1. Determine the number of points required for a competition curve and the desired number of replicates. To demonstrate a good competition curve, sixteen 3-fold dilutions, starting at 10 μ M, are recommended. Each well will receive 10 μ L of the unlabeled ligand titrations, and this amount does not account for "dead" volume in the pipetting technique (see above). Figure 1 illustrates a suggested plate format.
- Prepare the working dilution of the unlabeled NDP-α-MSH ligand to 2.5X the final assay concentration using 1X MC5 Binding Buffer *with 3% (w/v) MSD Blocker A added (30 mg/mL)*. Note: From this 2.5X working dilution, 10 µL/well will be added with a total assay volume of 25 µL/well yielding the final 1X ligand.
- Prepare 1:3 serial dilutions of unlabeled ligand fifteen times using the 1X MC5 Binding Buffer-3% Blocker A, including a zero-ligand final point.

Dilute labeled ligand:

- 1. Each well will receive $13 \ \mu L$ of labeled ligand for competition curves or $23 \ \mu L$ for saturation-binding curves. Remember to include calculations for extra ligand solution to account for the "dead" volume in pipetting technique.
 - a. For cold competition curves, dilute MSD TAG labeled NDP-α-MSH to 1.92 nM working concentration in 1X MC5 Binding Buffer with 3.0% (w/v) MSD Blocker A added (30 mg/mL). Note: The final assay concentration will be 1 nM labeled ligand.



Notes:

MSD MULTI-ARRAY plates are compatible with most binding buffers. A wide variety of binding buffers have been tested. The buffer supplied has been optimized for the biological reagents provided.

Unlabeled NDP- α -MSH sticks to labware. If preparing a competition curve, it is important to dilute into 3% MSD Blocker A to obtain accurate IC₅₀ values.

Labeled NDP- α -MSH and unlabeled NDP- α -MSH can be stored at 4 °C for up to 2 weeks. Ligands can be aliquoted and placed at -20 °C for long-term storage.

For other GPCR systems, it is recommended to optimize the concentration of Blocker A (added to the Binding Buffer) through a titration experiment. b. For a saturation-binding curve, eight, 2-fold serial dilutions starting at 16 nM are recommended to produce a complete curve. Prepare the working dilution of MSD TAG labeled NDP-α-MSH to 1.09X the final assay concentration in 1X MC5 Binding Buffer containing 3.0% (w/v) MSD Blocker A (30 mg/mL). Prepare 1:2 serial dilutions of labeled ligand seven times in 1X MC5 Binding Buffer-3% MSD Blocker A, including a zero-ligand final point. Note: From the 1.09X working dilutions, 23 µL/well will be added with a total assay volume of 25 µL/well yielding the final 1X ligand.

Dilute Read Buffer:

- 1. Determine the total number of wells to be used in the experiment. Each well will receive 10 µL of 3.5X Read Buffer.
- 2. Dilute 4X MSD Read Buffer T (surfactant-free) to 3.5X with deionized water (35 mL 4X Read Buffer T without surfactant per 40 mL total volume).

IV. Detailed Instructions:

Saturation-Binding Assay Protocol:

Begin with an MSD MULTI-ARRAY PEI 384 Plate (High Bind). No pre-treatment is necessary.

- 1. Carefully deliver 2 μ L/well of *quick-thawed, syringe-processed, and diluted* membranes directly to the center of the working electrode, taking care to ensure the droplet is contained to the electrode surface.
- 2. Seal or cover, and incubate at room temperature for 1 hour.
- 3. Dispense 23 μ L/well of the MSD TAG labeled NDP- α -MSH titrations.
- 4. Seal or cover, and incubate at room temperature for 1 hour.
- Dispense 10 µL/well of the diluted 3.5X MSD Read Buffer T (surfactant free) and analyze *immediately* with the SECTOR[™] Imager.

Competition-Binding Assay Protocol:

Begin with an MSD MULTI-ARRAY PEI 384 Plate (High Bind). No pre-treatment is necessary.

1. Carefully deliver 2 μ L/well of *quick-thawed, syringe-processed, and diluted* membranes directly to the center of the working electrode, taking care to ensure the droplet is contained to the electrode surface.



- 2. Seal or cover, and incubate at room temperature for 1 hour.
- Dispense 10 μL/well of the unlabeled NDP-α-MSH titrations. The plate may be incubated while stacked with a cover plate at room temperature for 1 hour to simulate HTS workflow inconsequential for demonstration purposes.
- 4. Dispense 13 μ L/well of diluted MSD TAG labeled NDP- α -MSH solution.
- 5. Seal or cover, and incubate at room temperature for 1 hour.
- Dispense 10 μL/well of diluted 3.5X MSD Read Buffer T (surfactant free) and analyze *immediately* with the SECTOR Imager.

Figure 1. Suggested plate format for saturation and competition binding curves using a MULTI-ARRAY 384 Plate.



