MSD® Cyclic AMP Assay Demonstration Kit

MULTI-ARRAY® 96-well Small Spot Plate

I. Materials Included

	Storage	Concentration
❑ MULTI-ARRAY anti-cAMP 96-well Small Spot Plate	2-8°C	N/A
❑ TAG™ Labeled cAMP	2-8°C	lyophilized
❑ Read Buffer T, with surfactant (4X) Tris-buffered solution containing an ECL co-reactant and Triton X-100, pH 7.8	RT	4X
❑ cAMP Assay Buffer HEPES buffered saline solution containing $MgCl_2$, pH 7.3	2-8°C	1X
❑ cAMP Lysis Buffer HEPES-buffered saline solution containing $MgCl_2$ and Triton X-100, pH 7.3	2-8°C	1X
❑ Blocker A A proprietary cocktail of proteins including BSA, optimized for use with MULTI-ARRAY plates.	RT	lyophilized
❑ cAMP Standard	≤-10°C	1 mM

II. Other Materials & Equipment (not supplied)

- ❑ Cells, cell culture supplies, compounds, etc.
- ❑ IBMX or other phosphodiesterase inhibitors.
- ❑ Hand pipettes, tubes, source plates, tips, etc. for making serial dilutions of standards. Required sizes will depend upon scale of experiment and desired throughput.
- ❑ Multi-channel pipetting equipment capable of accurately dispensing 10 µL, 20 µL, and 100 µL into a 96 well microplate.
- ❑ Adhesive plate sealers.

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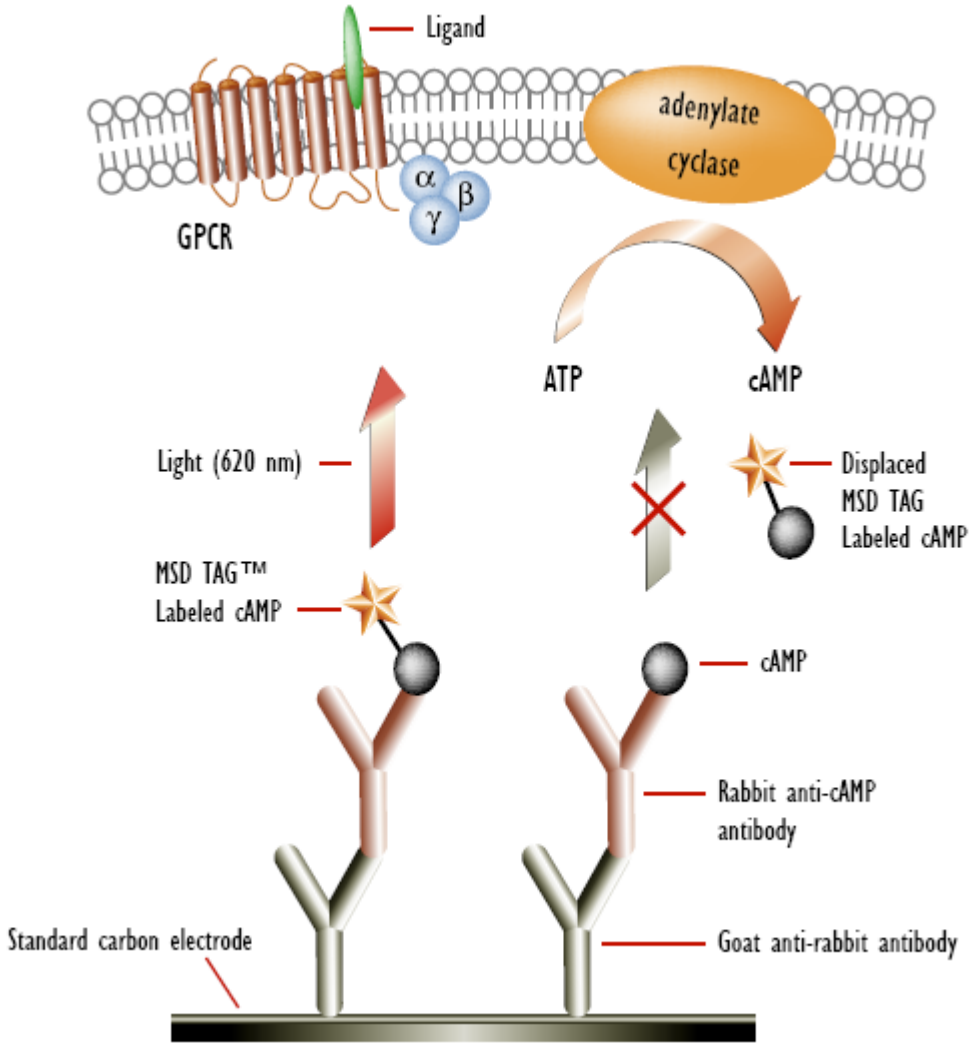
III. Principle of the Assay

The MSD cyclic-AMP assay is a competitive immunoassay based on the displacement of a cAMP molecule carrying an electrochemiluminescent label. In the absence of cAMP, a large proportion of the labeled cAMP is bound by an antibody on the surface of a disposable carbon electrode. Elevated concentrations of cAMP proportionally displace the labeled analog. When a potential is applied to the electrode, bound label produces light and a quantitative measure of cAMP is recorded.

The simple, no-wash protocol is suitable for HTS applications. GPCR activation studies can be performed in one hour, and a single user can process 150 plates in three hours. The assay displays robust performance when used to measure the activation or inhibition of GPCRs.

Figure 1. MSD cAMP Assay.



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IV. Protocol at a Glance

Standard Protocol: Stimulating cells and detecting cAMP in MSD plate

1. Add compound(s) or cAMP standards.
2. Add cells, incubate 30 minutes.
3. Add detection reagent, incubate 30 minutes to 2 hours.
4. Analyze plate.

Notes:

Read the entire detailed protocol below before beginning work.

V. Preparation & General Notes

- **Prior to starting assay, add 1 mg/mL Blocker A to Assay Buffer and Lysis Buffer.** *All references below are to buffers with Blocker A added.*

MSD MULTI-ARRAY plates are compatible with most assay buffers and cell culture media. A wide variety of solutions have been tested, including cell culture media.

- MSD TAG labeled cAMP is supplied lyophilized. Add 450 µL of Lysis Buffer to the vial to prepare a 500 nM stock solution. The amount of TAG-cAMP provided (225 pmol) is not visible. It is essential to thoroughly wash the sides of the vial with buffer to ensure proper TAG-cAMP resuspension. This 500 nM stock will be diluted 1:200 with cAMP Lysis Buffer in the assay. Keep on ice until use, then aliquot and store at -20 °C.

Reconstituted MSD TAG-cAMP may be stored at -20 ºC for longer than one year.

- To stimulate cells in the MSD cAMP plate:
    a. A cAMP standard titration should be prepared **5X** more concentrated than desired. To each well, 10 µL will be added. Assay Buffer may be used to serially dilute cAMP standards. A final concentration range of 10 µM to 0.2 nM is recommended. This can be achieved through a series of 11(1:3) dilutions. The 12th titration point should not include standard (0 µM). The IC_{50} is between 20-50 nM.
    b. The optimal number of cells will need to be determined for each application, however 20,000 cells in 20 µL is a recommended starting point. This can be achieved by the following steps: counting cells, pelleting them by centrifugation, decanting the supernatant, and resuspending the cells at 1.0 x 10^6 cells/mL with Assay Buffer (see Notes). A phosphodiesterase inhibitor such as 2.5 mM IBMX (not provided) should be added directly to the cells *immediately before* dispensing to the plate. The IBMX is then diluted to a final concentration of 1 mM in the well.
    c. Compounds (not provided) should be prepared **3X** more concentrated than desired as 10 µL will be added for a final volume of 30 µL at the point of action prior to lysis. A broad range of compound titrations is recommended to demonstrate a good curve.

Cells may also be prepared in most common media types or buffers.

It is not recommended to add IBMX directly to the cell dilution buffer since this will prematurely expose the cells to IBMX and elevate basal levels of cAMP.

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- To prepare Read Buffer:
    a. Approximately 20 mL per plate is required.
    b. Dilute 4X MSD Read Buffer T, with surfactant, to 1.5X. This can be done by adding 7.5 mL MSD Read Buffer T, with surfactant, to 12.5 mL deionized water.

Notes:

MULTI-ARRAY plates meet SBS standards and are easily interfaced with common liquid handling devices.

VI. Detailed Instructions

Standard Assay Protocol:

Begin with an MSD anti-cAMP coated MULTI-ARRAY 96-well Small Spot Plate. No pre-treatment is necessary.

1. Dispense 10 µL/well of **3X** compound(s) or 10 µL/well of **5X** cAMP standard titrations in Assay Buffer.

2. Dispense 20 µL/well of cells suspended in Assay Buffer. Testing several concentrations around $1.0x10^6$ cells/mL (20,000 cells/well) is recommended. Dispense the same volume of Assay Buffer only (no cells) to wells containing the cAMP standard titrations.

3. Incubate the plate sealed at RT for 30 minutes with gentle shaking. During this time prepare MSD TAG-cAMP.

4. Dilute TAG-cAMP to 2.5 nM (1:200) in Lysis Buffer and dispense 20 µL per well. The final concentration of TAG-cAMP will be 1 nM in the well.

5. Incubate the plate sealed at RT with shaking for 30 minutes to 2 hours. Signals will generally increase with extended incubation times up to 2 hours.

6. Dispense 100 µL of 1.5X Read Buffer T to each well.

7. Analyze the plate *immediately* with an MSD instrument.

Shaking a 96-well MULTI-ARRAY or MULTI-SPOT® plate accelerates capture at the working electrode.

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