CD39 Inhibitor Screening Assay Kit

#79278
96 reactions

Description
The CD39 Inhibitor Screening Assay Kit is a colorimetric assay designed to measure the activity of CD39 for screening and profiling applications. The kit comes in a convenient 96-well format with enough purified CD39 enzyme, ATP, CD39 assay buffer, and colorimetric detection reagent for 100 enzyme reactions. In addition, the kit includes the CD39 inhibitor POM-1 for use as an inhibitor control.

Background
B cell activation marker Cluster of Differentiation 39 (CD39), also known as ecto-apyrase, ATP diphosphohydrolase, ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1), hydrolyzes extracellular nucleotide tri- and diphosphates into AMP in the presence of Ca^{2+} and Mg^{2+}. It is an important enzyme in many biological processes, including the modulation of neural cell activities, prevention of intravascular thrombosis, and regulation of immune responses. The role of regulatory T cells (Treg) links closely to CD39/CD79, and a dysfunction in this pathway can lead to cancer progression. The use of CD39 inhibitors can prove advantageous for the treatment of pathologies where CD39/CD73 pathways play a role.

Applications
Study enzyme kinetics and screen small molecule inhibitors for drug discovery and High Throughput Screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71284	CD39, His-Tag*	1 µg	-80°C
79279	4x CD39 Assay Buffer	3 ml	-20°C
	35 mM ATP	15 µl	-20°C
	POM-1, MW=2986.01 g/mol**	1.5 mg	-20°C
74001	Colorimetric Detection Reagent***	10 ml	4°C
79963	Transparent 96-well microtiter plate	1	Room Temp.

**The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*
***CD39 inhibitor POM-1 is provided as a control for CD39 inhibition.*
****Colorimetric Detection Reagent is used to measure the free phosphate generated in the reaction catalyzed by CD39. Any other source of inorganic phosphate will interfere with the assay.*

Materials Required but Not Supplied

Name	Catalog #
UV/Vis spectrophotometer microplate reader capable of reading λ=630 nm	
Adjustable micropipettor and sterile tips	

Stability
This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

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Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw **4x CD39 Assay Buffer** and **35 mM ATP** on ice.

2. Prepare a master mix (10 µl/well): N wells x (5 µl of 4x CD39 Assay Buffer + 5 µl of distilled water).

3. Add 10 µl of master mix to all wells.

4. Prepare **1x CD39 Assay Buffer** by diluting 4-fold CD39 Assay Buffer in distilled water. 6 ml of 1x CD39 Assay Buffer is enough for 100 reactions. Dilute only enough buffer required for the assay. Store the remaining 4-fold CD39 Assay Buffer in single use aliquots at -20°C.

5. Add 20 µl of 1x CD39 Assay Buffer to the "Blank" well.

6. Prepare Test Inhibitor (10 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   6.1. If the test inhibitor is soluble in water, make a dilution in 1x CD39 Assay buffer at a concentration 5-fold higher than the final desired concentration. The 1x CD39 Assay Buffer is the Diluent Solution.

   OR

   6.2. If the Test Inhibitor is soluble in DMSO, dissolve in 100% DMSO at a concentration 100-fold higher than the highest desired concentration. Then make a 20-fold dilution in 1x CD39 Assay Buffer. The compound concentration is 5-fold higher than the final desired concentration and the DMSO concentration is 5%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in CD39 Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% DMSO in 1x CD39 Assay Buffer so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO in the assay should not exceed 1%.

7. Add 10 µl of Test Inhibitor to the wells labeled "Test Inhibitor".

8. Add 10 µl of Diluent Solution to the "Blank" and "Positive Control" wells.

9. Dissolve 1.5 mg of POM-1 in 500 µl of 1x CD39 Assay Buffer to obtain a 1 mM solution.

10. Add 10 µl of 1 mM POM-1 solution to the "Inhibitor Control" wells. Aliquot and store remaining solution at -80°C.

11. Thaw **CD39** on ice. Briefly spin the tube to recover the full content of the tube.

12. Dilute CD39 to 0.25-0.3 ng/µl with 1x CD39 Assay Buffer (20 µl/well). If the assay plate is going to be used more than once, prepare enough enzyme for this portion of the assay and aliquot the remaining undiluted protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

    Note: CD39 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not reuse thawed aliquots or diluted enzyme.

13. Add 20 µl of diluted CD39 to the "Positive control", "Test Inhibitor" and "Inhibitor Control" wells.

14. Cover the plate with foil and incubate for 30 minutes at room temperature with gentle agitation.

15. During the incubation period prepare a diluted ATP solution by diluting 35 mM ATP 100-fold with 1x CD39 Assay Buffer (10 µl/well). Prepare only the amount required for the assay and store the remaining 35 mM ATP in single use aliquots at -20°C. Discard unused diluted ATP solution.

Component	**Blank**	**Positive Control**	**Test Inhibitor**	**Inhibitor Control**
Master Mix	10 µl	10 µl	10 µl	10 µl
1x CD39 Assay Buffer	20 µl	-	-	-
Test Inhibitor	-	-	10 µl	-
1 mM POM-1	-	-	-	10 µl
Diluent Solution	10 µl	10 µl	-	-
CD39 (0.25-0.3 ng/µl)	-	20 µl	20 µl	20 µl
Incubate 30 minutes at room temperature				
ATP (diluted)	10 µl	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

16. Initiate the reaction by adding 10 µl diluted ATP to all the wells.

17. Incubate at room temperature for 30 minutes.

18. Add 100 µl of Colorimetric Detection Reagent to all wells.

19. Cover the plate with foil and incubate the plate for 15 minutes at room temperature.

20. Measure the absorbance in a plate reader at λ=630 nm.

21. Subtract the "Blank" value from all other values (background value).

Example of Assay Results

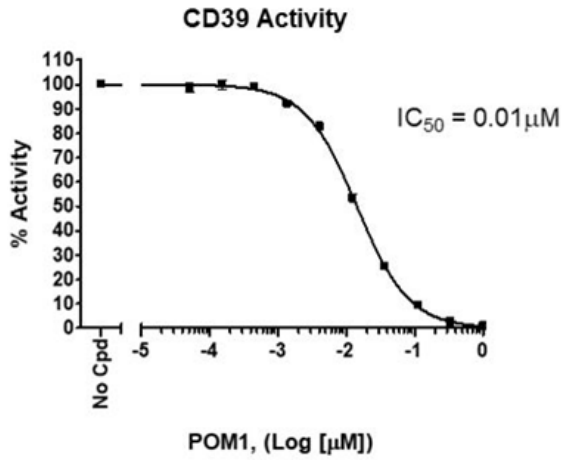

Figure 1: Inhibition of CD39 enzyme activity by POM-1.
CD39 activity was measured in the presence of increasing concentrations of inhibitor POM-1. Absorbance was measured using a Tecan Infinite M1000 microplate reader. Results are expressed as percent of control activity (measured in the absence of POM-1 and set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General considerations
"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Guo W, *et al*., CD39- A bright target for cancer immunotherapy, *Biomed Pharmacother.,* 2022*;* 151: 113066.

Related Products

Products	*Catalog #*	*Size*
CD39, His-Tag (Human) Recombinant	71284	50 µg
CD38, His-Tag (Human), HIP™ Recombinant	71277	100 µg
CD73, His-Tag (Human) Recombinant	71184	50 µg/500 µg
CD73, Avi-His-Tag (Mouse) Recombinant	72523	25 µg/100 µg
CD38 Inhibitor Screening Assay Kit (Cyclase Activity)	71275	96 reactions
CD73 Inhibitor Screening Assay Kit	72055	96 reactions
CD73 Inhibitor Screening Assay Kit	72058	384 reactions