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Data Sheet

CD39 Inhibitor Screening Assay Kit

Catalog: 79278
Size: 96 reactions

BACKGROUND: B cell activation marker CD39, also known as ecto-apyrase, ATP diphosphohydrolase, ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1) hydrolyzes extracellular nucleotide tri- and diphosphates in the presence of Ca++ and Mg++. 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.

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

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71284	CD39	1 µg	-80°C	***Avoid multiple freeze/thaw cycles!***
79279	4x CD39 Assay Buffer	3 ml	-20°C	
	ATP (35 mM)	15 µl	-20°C	
	POM-1	1.5 mg	-20°C	
74001	Colorimetric Detection Reagent*	10 ml	+4°C	
	Transparent 96-well plate	1	Room Temp.	

**Colorimetric Detection Reagent is used to measure the free phosphate from the CD39 reaction. Any source of inorganic phosphate can interfere with the assay.*

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 630 nm
Adjustable micropipettor and sterile tips
Rotating or rocker platform (optional)
Aluminum foil

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

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REFERENCE: Wang, T.-F., *et al.*, *J. Biol. Chem.* **273 (38),** 24814-21 (1998)

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1. Thaw **4x CD39 assay buffer** and **ATP** on ice.

2. Prepare the master mixture (10 µl per well): N wells x (5 µl **4x CD39 assay buffer** + 5 µl distilled water). Add 10 µl to every well.

	Positive Control	Test Inhibitor	Blank
4x CD39 assay buffer	5 µl	5 µl	5 µl
Water	5 µl	5 µl	5 µl
Test Inhibitor	–	10 µl	–
Inhibitor Buffer (no inhibitor)	10 µl	–	10 µl
1x CD39 assay buffer	–	–	20 µl
CD39 (0.25-0.3 ng/µl)	20 µl	20 µl	–
ATP (diluted)	10 µl	10 µl	10 µl

3. Add 10 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the wells labeled “Positive Control" and “Blank”, add 10 µl of the same solution without inhibitor (Inhibitor buffer).

4. To make a 1 mM stock of the standard inhibitor, add 500 µl water to the tube containing 1.5 mg of **POM-1**.

5. Prepare **1x CD39 assay buffer** by diluting **4x CD39 assay buffer** with water. Dilute only enough buffer required for the assay. Store remaining **4x CD39 assay buffer** at -20°C in single-use aliquots. For 100 reactions, prepare 6 ml **1x CD39 assay buffer** by mixing 1.5 ml of **4x CD39 assay buffer** with 4.5 ml water.

6. To the wells designated as "Blank", add 20 µl of **1x CD39 assay buffer.**

7. Thaw **CD39** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **CD39** required for the assay and dilute enzyme to 0.25-0.3 ng/µl with **1x CD39 assay buffer** (5-6 ng/well). Aliquot remaining **CD39** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **CD39** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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8. Add 20 µl of diluted **CD39** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Cover the plate and incubate 30 minutes at room temperature with slow shaking.

9. During the incubation, dilute **ATP** 100-fold with **1x CD39 assay buffer**. Dilute only the amount required for the assay. Store remaining **ATP** at -20°C in single use aliquots. Discard any unused diluted **ATP** after use.

10. After the 30 minute incubation, remove the plate.

11. Initiate the reaction by adding 10 µl of diluted **ATP** to the wells designated “Positive Control”, "Test Inhibitor Control" and “Blank”. **Incubate at room temperature for 30 minutes**.

12. After the reaction, add 100 µl of **Colorimetric Detection Reagent** into each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 15 minutes. During the 15 minute incubation, the plate can be placed on a rocker platform (optional).

13. Set the microplate reader and read Absorbance at 630 nm. Subtract “Blank” value from all other values.

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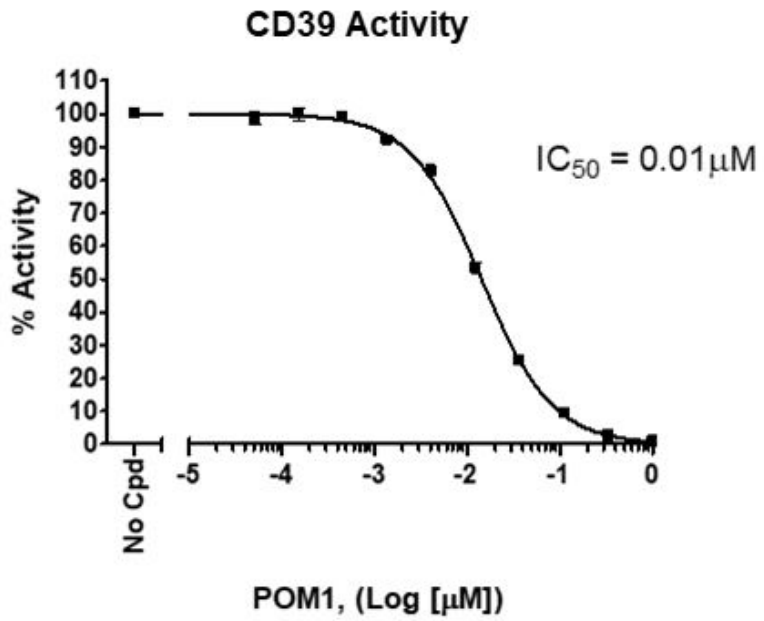
Example of Assay Results:



CD39 inhibition by POM-1, measured using the CD39 Inhibitor Screening Assay Kit, BPS Bioscience Cat. 79278. The absorbance at 630 nm was measured using a Tecan Infinite M1000 microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
CD39, His-tag	71284	20 µg
CD38, His-tag	71277	100 µg
CD38-APC, His-Tag	71883	100 µg
CD73, His-tag	71184	50 µg
CD73, Avi, His-tag (Mouse)	72523	100 µg
CD38 Inhibitor Screening Assay Kit	71275	96 rxns.
CD73 Inhibitor Screening Assay Kit	72055	96 rxns.
CD73 Inhibitor Screening Assay Kit	72058	384 rxns.
5X CD73 Assay Buffer	74000	10 ml