CD73 Inhibitor Screening Assay Kit

#72055
96 reactions

Description
The CD73 Inhibitor Screening Assay Kit is a 96-well colorimetric assay designed to measure the activity of CD73 for screening and profiling applications. The kit contains enough purified CD73 enzyme (BPS Bioscience #71184), AMP, CD73 assay buffer and colorimetric detection reagent for 100 enzyme reactions.

Background
Cluster of Differentiation 73 (CD73), also known as ecto-5'-nucleotidase (NT5E) degrades AMP into adenosine. Adenosine is an anti-inflammatory molecule, and its regulation is crucial for immune system homeostasis. CD73 is expressed in several cell types, such as Treg cells and myeloid-derived suppressor cells. Upregulation of CD73 is linked to numerous cancer types, such as leukemia, glioblastoma, melanoma and ovarian and breast cancer. It is also linked to systemic lupus erythematosus. Its role in cancer makes CD73 inhibitors and antibodies promising therapeutical avenues for cancer and inflammatory diseases.

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

Supplied Materials

Catalog #	Name	Amount	Storage
71184	CD73, His-Tag*	5 µg	-80°C
74000	5x CD73 Assay Buffer	2 x 1 ml	-20°C
79496	500 µM AMP	1 ml	-20°C
74001	Colorimetric Detection Reagent**	10 ml	4°C
79963	Transparent 96-well plate	1	Room Temp.

**The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*
***Colorimetric Detection Reagent is used to measure the free phosphate generated in the reaction catalyzed by CD73. Any other source of inorganic phosphate can interfere with the assay.*

Materials Required but Not Supplied
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.

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.

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Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw **5x CD73 Assay Buffer** and **500 µM AMP** on ice.

2. Prepare a master mix (25 µl/well): N wells x (6 µl 5x CD73 Assay Buffer + 10 µl 500 µM AMP + 9 µl distilled water). Store the remaining 500 µM AMP in single use aliquots at -20°C.

3. Add 25 µl of master mix to each well.

4. Prepare **1x CD73 Assay Buffer** by diluting 5-fold 5x CD73 Assay Buffer with distilled water. 3 ml of 1x CD73 Assay Buffer is enough for 100 reactions. Dilute only enough buffer required for the assay. Store the remaining 5x CD73 Assay Buffer in single use aliquots at -20°C.

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

   5.1. If the test inhibitor is soluble in water, make a dilution in 1x CD73 Assay buffer at a concentration 10-fold higher than the final desired concentration. The 1x CD73 Assay Buffer is the Diluent Solution.
   OR
   5.2. 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 10-fold dilution in 1x CD73 Assay Buffer. The compound concentration is 10-fold higher than the final desired concentration and the DMSO concentration is 10%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in CD73 Assay Buffer to keep the concentration of DMSO constant.
   For positive and negative controls, prepare 10% DMSO in 1x CD73 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%.

6. Add 5 µl of Test Inhibitor to the wells labeled “Test Inhibitor”.

7. Add 5 µl of Diluent Solution to the “Blank” and “Positive Control” wells.

8. Add 20 µl of 1x CD73 Assay Buffer to the “Blank” well.

9. Thaw **CD73** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

10. Dilute CD73 in 1x CD73 Assay Buffer to 0.05-0.1 ng/µl. You need 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: CD73 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not reuse thawed aliquots or diluted enzyme.

11. Start the reaction by adding 20 µl of diluted CD73 to the "Positive Control" and "Test Inhibitor" wells.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x CD73 Assay Buffer	20 µl	-	-
CD73 (0.05-0.1 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Incubate at 37°C for 25 minutes.

13. Add 100 µl of Colorimetric Detection Reagent.

14. Cover with foil and incubate for 15 minutes at Room Temperature.

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

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

Example of Assay Results

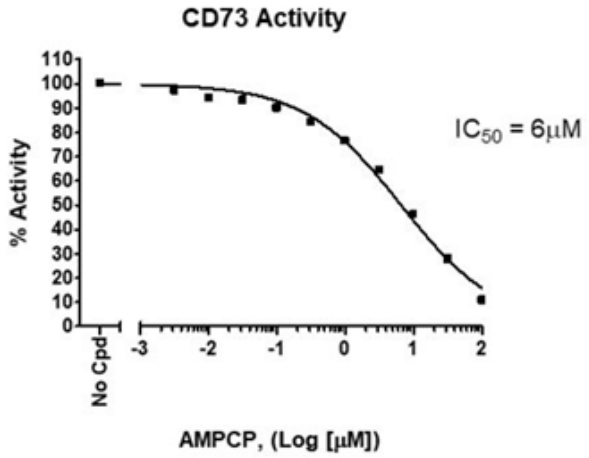

Figure 1: Inhibition of CD73 activity by AMPCP.
CD73 activity was measured in the presence of increasing concentrations of inhibitor AMPCP. Absorbance was measured using a Bio-Tek microplate reader. Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

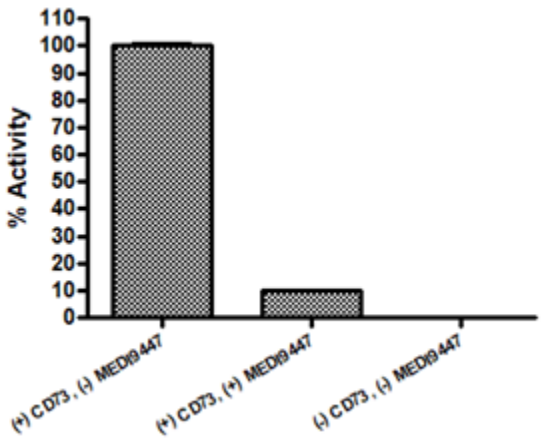

Figure 2: Inhibition of CD73 by an anti-CD73 antibody.
CD73 was preincubated with the antibody (1:1 molar ratio) for 1 hour at room temperature. The reaction was initiated by adding AMP and conducted for 20 minutes at 37°C. Results are expressed as percent of control activity (measured in the absence of antibody 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
1. Antonioli L., *et al*., 2013, CD39 and CD73 in immunity and inflammation, *Trends Mol Med*., 19 (6): 355-67.
2. Antonioli L., *et al*., 2013, Immunity, inflammation and cancer: a leading role for adenosine, *Nat Rev Cancer*. 13 (12) : 842-57.
3. Roh M., *et al.*, 2020, Targeting CD73 to augment cancer., *Immunotherapy Curr Opin Pharmacol.* 53 : 66-76.

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
CD39 Inhibitor Screening Assay Kit	79278	96 reactions
CD73 Inhibitor Screening Assay Kit	72058	384 reactions