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Data Sheet

CD73 Inhibitor Screening Assay Kit

Catalog: #72058
Size: 384 reactions

BACKGROUND : CD73, also known as Ecto-5'-nucleotidase (5'-NT), promotes tumor growth and progression by degrading AMP into adenosine. CD73-generated adenosine blocks T-cell immuno-surveillance, resulting in an immunosuppressed and pro-angiogenic niche within the tumor microenvironment.

DESCRIPTION: The *CD73 Inhibitor Screening Assay Kit* is designed to measure CD73 activity for screening and profiling applications. The CD73 assay kit comes in a convenient 384-well format, with purified recombinant CD73 enzyme, AMP, CD73 assay buffer, and Colorimetric detection reagent for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71184	CD73	5 µg	-80°C	***Avoid multiple freeze/thaw cycles!***
74000	5x CD73 assay buffer	3 x 1 ml	-20°C	
79496	AMP (500 µM)	2 x 1 ml	-20°C	
74001	Colorimetric detection reagent*	2 x 10 ml	+4°C	
79962	Transparent 384-well plate	1	Room Temp.	

**Colorimetric detection reagent is used to measure the free phosphate from the CD73 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
37˚C incubator
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.

REFERENCE: Antonioli, L., *et al.*, *Trends in Cancer*, Vol. 2, No. 2, 95-109.

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

1. Thaw **5x CD73 assay buffer** and **AMP** on ice.

2. Prepare the master mixture (12.5 µl per well): N wells x (3 µl **5x CD73 assay buffer** + 5 µl **AMP**(500 µM) + 4.5 µl water). Add 12.5 µl to every well. Store remaining **AMP** at -20°C in single use aliquots.

	Positive Control	Test Inhibitor	Blank
5x CD73 assay buffer	3 µl	3 µl	3 µl
AMP (500 µM)	5 µl	5 µl	5 µl
Water	4.5 µl	4.5 µl	4.5 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor Buffer (no inhibitor)	2.5 µl	–	2.5 µl
1x CD73 assay buffer	–	–	10 µl
CD73 (0.02—0.03 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3. Add 2.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the wells labeled “Positive Control" and “Blank”, add 2.5 µl of the same solution without inhibitor (Inhibitor buffer).

4. Prepare **1x CD73 assay buffer** by diluting **5x CD73 assay buffer** with water. Dilute only enough buffer required for the assay. Store remaining **5x CD73 assay buffer** at -20°C in single-use aliquots. For 100 reactions, prepare 5 ml **1x CD73 assay buffer** by mixing 1 ml of **5x CD73 assay buffer** with 4 ml water.

5. To the wells designated as "Blank", add 10 µl of **1x CD73 assay buffer.**

6. Thaw **CD73** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **CD73** required for the assay and dilute enzyme to ~ 0.02 - 0.03 ng/µl with **1x CD73 assay buffer**. Aliquot remaining **CD73** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: We recommend diluting **CD73** in multiple steps because of the small concentration needed for testing. **CD73** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7. Initiate reaction by adding 10 µl of diluted **CD73** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". **Incubate at 37°C for 20 minutes**.

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8. After the 20 minute reaction, remove the plate and add 50 µl of **Colorimetric detection reagent**. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes depending on the reaction progress. Multiple reading at every 5 min can be done to have good S/B window. During the incubation, the plate can be placed on a rocker platform (optional).

9. Set the microplate reader and read Absorbance at 630 nm. Subtract "Blank" value from all other values.

Example of Assay Results:

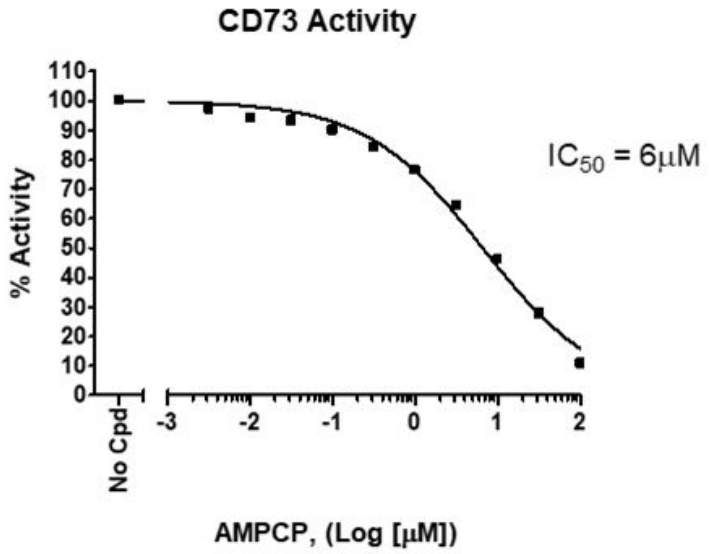

Figure 1. CD73 activity (left) and its inhibition by AMPCP (right), measured using the CD73 Colorimetric Activity Assay Kit, BPS Bioscience Cat. # 72055. Absorbance was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

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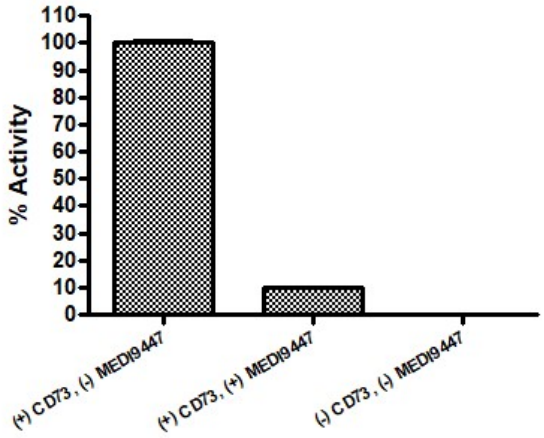

Figure 2. Inhibition of CD73 by an anti-CD73 antibody. Experiment was done by following the assay kit procedure (above) with a small modification. CD73 was preincubated with the antibody (1:1 molar ratio) for 1 hour at room temperature and the reaction was initiated by adding AMP and conducted for 20 min at 37°C.

RELATED PRODUCTS:

Product Name	Catalog #	Size
CD73 Inhibitor Screening Assay Kit	72055	384 rxns
5'-Nucleotidase/CD73, His-tag	71184	50 µg
Adenosine Deaminase (ADA), His-tag	70016	100 µg
TCF/LEF Reporter Kit	60500	500 rxns
TCF/LEF reporter-HEK293 cell line	60501	2 vials