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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      | <b><i>Avoid multiple freeze/thaw cycles!</i></b> |
| 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       |  |
|           | 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  $\mu$ l per well): N wells x (3  $\mu$ l **5x CD73 assay buffer** + 5  $\mu$ l **AMP**(500  $\mu$ M) + 4.5  $\mu$ l water). Add 12.5  $\mu$ l to every well. Store remaining **AMP** at -20°C in single use aliquots.

|                                 | Positive Control | Test Inhibitor | Blank       |
|---------------------------------|------------------|----------------|-------------|
| 5x CD73 assay buffer            | 3 $\mu$ l        | 3 $\mu$ l      | 3 $\mu$ l   |
| AMP (500 $\mu$ M)               | 5 $\mu$ l        | 5 $\mu$ l      | 5 $\mu$ l   |
| Water                           | 4.5 $\mu$ l      | 4.5 $\mu$ l    | 4.5 $\mu$ l |
| Test Inhibitor                  | -                | 2.5 $\mu$ l    | -           |
| Inhibitor Buffer (no inhibitor) | 2.5 $\mu$ l      | -              | 2.5 $\mu$ l |
| 1x CD73 assay buffer            | -                | -              | 10 $\mu$ l  |
| CD73 (0.02 – 0.03 ng/ $\mu$ l)  | 10 $\mu$ l       | 10 $\mu$ l     | -           |
| Total                           | 25 $\mu$ l       | 25 $\mu$ l     | 25 $\mu$ l  |

3. Add 2.5  $\mu$ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the wells labeled "Positive Control" and "Blank", add 2.5  $\mu$ l 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  $\mu$ l 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/ $\mu$ l 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  $\mu$ l 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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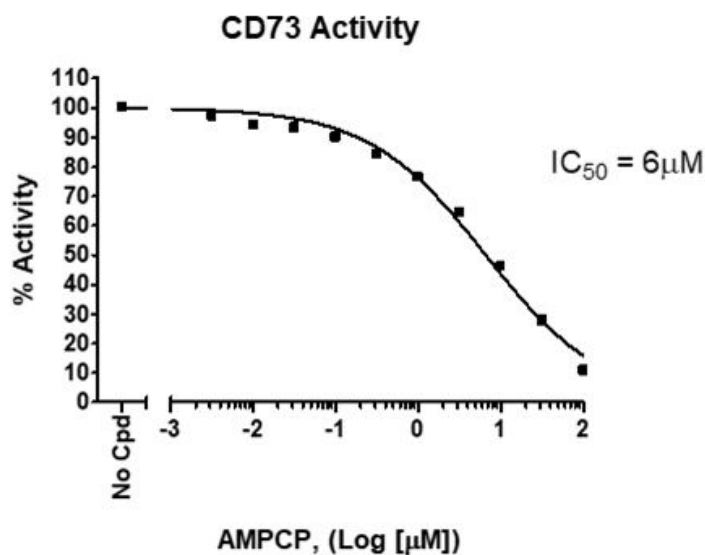
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- After the 20 minute reaction, remove the plate and add 50  $\mu$ l of **Colorimetric detection reagent**. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes depending on the reaction progress. Multiple reading 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).
- Set the microplate reader and read Absorbance at 630 nm. Subtract "Blank" value from all other values.

#### Example of Assay Results:



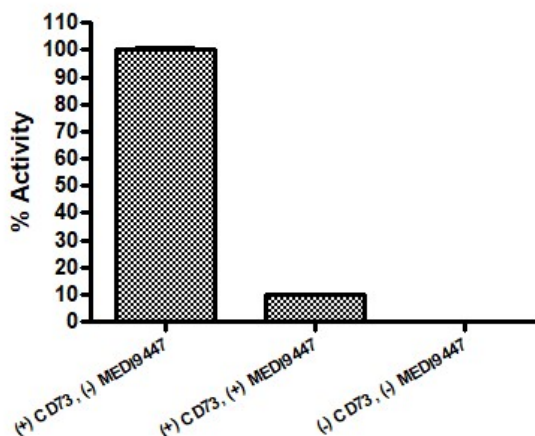
**Figure 1.** CD73 activity (left) and its inhibition by AMPCP (right), measured using the CD73 Colorimetric Activity Assay Kit, BPS Bioscience Cat. # 72055. Absorbance was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience,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

| <u>Product Name</u>                | <u>Catalog #</u> | <u>Size</u> |
|------------------------------------|------------------|-------------|
| 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     |

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