



# SZABO SCANDIC

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## Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

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### Lieferung & Zahlungsart

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### Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

### SZABO-SCANDIC HandelsgmbH

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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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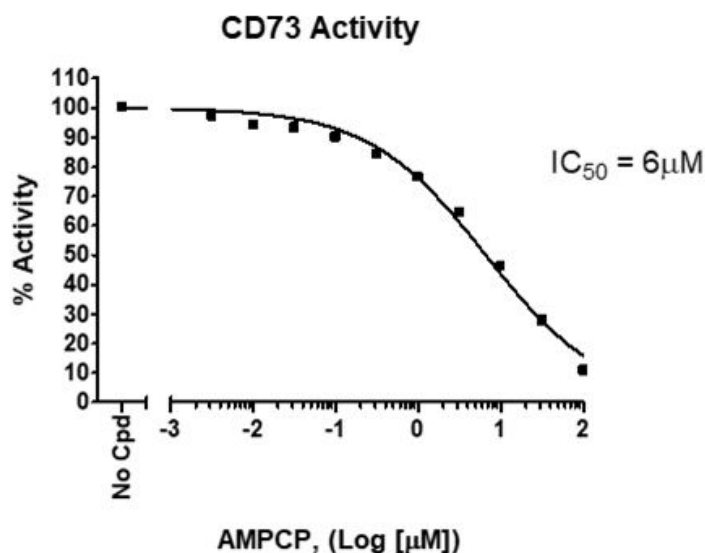
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- After the 20 minute reaction, remove the plate and add 50  $\mu$ l of **Colorimetric detection reagent**. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes depending on the reaction progress. Multiple reading 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:



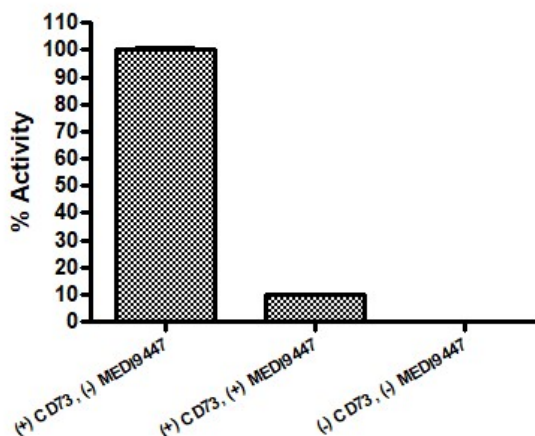
**Figure 1.** CD73 activity (left) and its inhibition by AMPCP (right), measured using the CD73 Colorimetric Activity Assay Kit, BPS Bioscience Cat. # 72055. Absorbance was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience,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