

5'-Nucleotidase (CD73) Activity Colorimetric Assay Kit (TBS2046, 100 Assays, Store at -20 °C)

Description

5'-Nucleotidase (5'-NT), also known as CD73 is an enzyme located in the plasma membrane. It converts extracellular nucleotides like 5'-AMP to their corresponding nucleosides, through phosphorylate cleavage. This conversion facilitates uptake of the nucleosides through nucleoside receptors into the cell, where they can again be phosphorylated to generate nucleotides and contribute to the nucleotide pool inside the cell. 5'-NT levels are elevated in hepatic diseases such as viral hepatitis, alcoholic liver disease and cirrhosis.

Tribioscience's CD73 Activity Kit provides a simple, easy assay that relies on the quantification of free phosphate with the Malachite Green complex. In this assay, the action of CD73 on the substrate generates free phosphate, which combines malachite green to form color complex. The color intensity is quantified at 620 nm. The assay kit also includes CD73 enzyme as positive control.

Applications

- Measure CD73 activity in a variety of samples.
- Screen CD73 inhibitors.

Kit Contents for 100 tests:

Name	Size (100 tests)
CD73 Standard (1mM)	200 μ L
CD73 Assay Buffer	25 mL
CD73 substrate	2.5 mL
CD73 Inhibitor	150 μ L
CD73 Color Probe	250 μ L
CD73 Positive Control	100 μ L

Storage conditions: Store the Reagent at -20°C, protect from light.
Shelf life: 12 months.

Sample Preparation

1. Sample protein is prepared with a suitable method and protein concentration is determined using preferred method.
2. Protein concentration should range between 1-10 mg/mL. Concentrated samples may be diluted with Assay Buffer.
3. Aliquot and store lysates at -80°C unless being used immediately.

Assay Procedures

Equilibrate all the kit components until room temperature before starting the experiment.

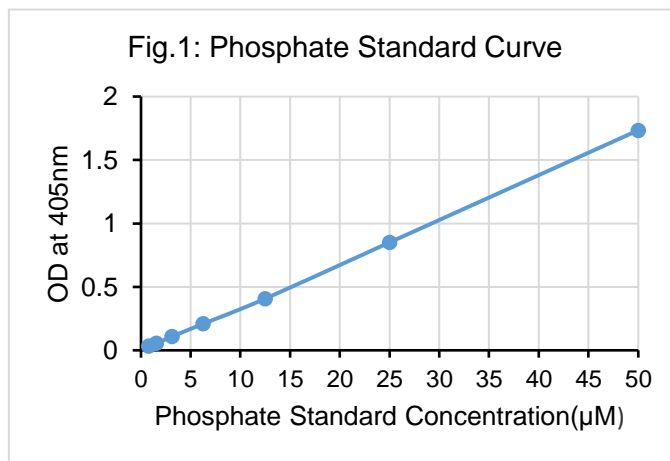
1. Prepare three identical wells for each sample labelled "Sample Background Control (BC), Sample (S), and Sample + Inhibitor (SI).
2. For SI well, add 5 μ L of CD73 Inhibitor in addition to samples.
3. For BC well, do not add Substrate, use Assay Buffer replace the substrate.
4. For positive control: add 5 μ L CD73 Enzyme stock and 45 μ L Assay Buffer as positive control.

5. Prepare the CD73 standards: Add 15 μ L of 1m M CD73 to 285 μ L of assay buffer in Tube #1, and then make a 2-fold serial dilution from Tube#2 to Tube#7 as the Table 1. Tube#8 as blank control.

Table 1: Standard Preparation

Tube #	CD73 Standard (μ L)	Assay Buffer (μ L)	CD73 Concentration (μ M)
1	15	285	50
2	150	150	25
3	150	150	12.5
4	150	150	6.25
5	150	150	3.13
6	150	150	1.56
7	150	150	0.78
8	0	150	0

6. Add 50 μ L sample or standard or positive control to each well.
7. Add 15 μ L CD73 Substrate to sample wells. Do not add it to standard and BC wells.
8. Adjust the volume of each well is 70 μ L with Assay Buffer.
9. Add 30 μ L CD Color Probe to each well.
10. Incubate at 37 °C for 20 min.
11. Read plate at 620 nm (600-660nm).
12. The typical CD73 standard curve is shown in Fig. 1.



Calculate CD73 activity

Based on the standard curve, the equation is gotten as below:

Equation 1: $Y = A * X + B$

The CD73 amount X (μ M) = $(Y - B) * DF / A$

Y = (Sample OD - Blank OD); A = Slope;

B = constant value; DF = dilution factor.

Equation 2:

Total CD73 Activity (μ M /min/mg) = $X / (\Delta T \times P)$

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Specific CD73 Activity in sample = Total CD73 Activity - CD73 inhibitor

X: CD73 amount

ΔT : Reaction time (i. e. 20 minutes per the procedure).

P: sample protein content added (mg).

Unit Definition: One unit of CD73 is the amount of enzyme that generates 1.0 μMol of Phosphate per minute at pH7.4 at 37°C.

RELATIVE PRODUCTS

CD38 Cyclase Activity Assay (TBS2100)
L-Lactate colorimetric assay (TBS2071)
LDH Cytotoxicity Assay (TBS2002)
Resazurin Cell Viability (TBS2001)
CCK-8 Cell Viability Assay (TBS2022)
Homocysteine Fluorometric Assay (TBS2091)
AHCY Inhibitor Screening Assay (TBS2097)
G6PDH Activity Colorimetric Assay (TBS2102)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric / Fluorometric Assay (TBS2020)
Caspase-3 Colorimetric Assay (TBS2030)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Assay (TBS2098)

This product is for research use only.