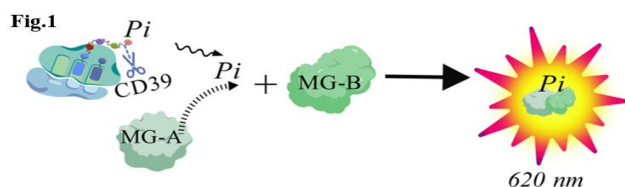


CD39/ENTPD1 Activity Colorimetric Assay Kit (TBS2126, 100 Assays, Store at -20 °C)

Description

Cluster of Differentiation 39 (**CD39/NTPDase-1**) is a cell-surface ectonucleotidase prominently expressed on leukocytes and endothelial cells. By catalyzing the hydrolysis of extracellular ATP to ADP and AMP, CD39 serves as a critical regulator of purinergic signaling in cancer, inflammation, and immunological disorders. Consequently, its dysregulation is heavily implicated in neuroinflammation, cardiac dysfunction, obesity, and reproductive diseases.

Tribioscience's CD39 Activity Kit provides a simple, easy assay that relies on quantification of free phosphate with Malachite Green complex. In this assay, CD39 in the presence of substrate generates free phosphate (Pi), which combines with malachite green reagents A (MG-A) and B (MG-B) to form the color complex shown in Fig. 1. The color intensity can be measured at 620 nm.



Target Synonym: ENTPD1, NTPDase-1, ATPDase, Ecto-ATPDase 1, Ecto-ATPase 1, Ecto-apyrase, ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1).

Applications

Measure CD39 activity in a variety of samples.

Kit Contents for 100 tests:

Part Name	Part Size
CD39 Standard (400 μM)	0.2 mL
CD39 Assay Buffer	10 mL
CD39 Substrate	70 μL (10x)
CD39 Reagent A	1.3 mL
CD39 Reagent B	0.4 mL
CD39 Positive Control	12 μL (10x)

Store the Reagent at -20°C, protected from light, for 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. Sample should be used in assay buffer.
4. Aliquot and store lysates at -80°C unless being used immediately.

Assay Procedures

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

1. Prepare the CD39 standards: Label Tube number #1 to #8. Add 100 μL of dd H₂O from Tube #1 through to #7, add 200 μL of dd H₂O to #8.

2. Make a 2-fold serial dilution by adding 100 μL of CD39 standard to the next concentration tube to make standard concentration from 200 to 3.125 μM shown in Table 1. Tube #8 as standard 0.

Table 1: CD 39 Standard Preparation

Tube	CD 39 standard (μL)	dd H ₂ O (μL)	Standard (μM)
1	100 μL of 400 μM	100	200
2	100 μL of 200 μM	100	100
3	100 μL of 100 μM	100	50
4	100 μL of 50 μM	100	25
5	100 μL of 25 μM	100	12.5
6	100 μL of 12.5 μM	100	6.125
7	100 μL of 6.25 μM	100	3.125
8	0	200	0

3. Preparing CD39 positive control working solution by adding 10 μL positive control (10x) to 90 μL of assay buffer.
4. Preparing CD39 substrate working solution by adding 60 μL of CD39 substrate (10x) to 540 μL of assay buffer.
5. Add 25 μL standards, samples, positive control working solution and blank (assay buffer) in the appropriate wells.
6. Add 5 μL of CD39 Substrate working solution to all wells. Mixed thoroughly.
7. Cover the plate and incubate at 37°C for 30 minutes with shaking.
8. Add 12 μL of CD39 Reagent A in each well. Then add 3 μL CD39 Reagent B in each well. Gently mixed thoroughly. Incubate 1-3 min at room temperature.
9. Read plate at 620 nm (absorbance).

Calculating CD39 activity

Based on the CD39 standard curve, the equation is expressed as below:

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

The CD39 amount X (μM) = $(Y - B) * DF / A$

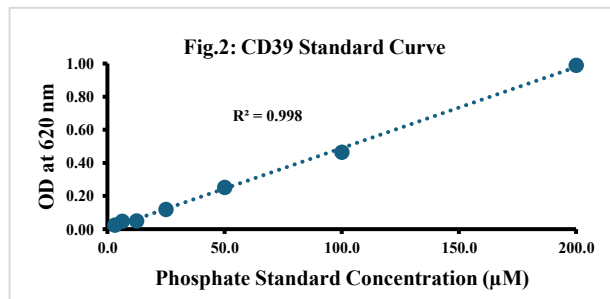
$Y = (\text{Sample OD} - \text{Blank OD}); A = \text{Slope};$

$B = \text{constant value}; DF = \text{dilution factor}.$

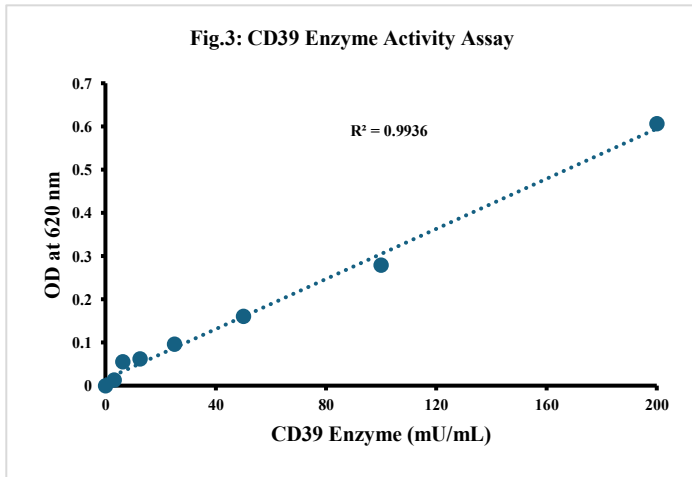
Equation 2:

Total CD39 Activity (μM /min) = $X / (\Delta T)$

Unit Definition: One unit of CD39 is the amount of enzyme that generates 1.0 μMol of Phosphorate per minute at pH7.5 at 37°C. The typical CD39 standard curve is shown in Fig. 2.



The typical CD39 activity assay is shown in Fig.3.



RELATIVE PRODUCTS

CD38 Cyclase Activity Assay (TBS2100)
 CD73 Activity Colorimetric Assay (TBS2046)
 CD203a Activity Colorimetric Assay (TBS2113)
 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 Activity Assay (TBS2098)
 Caspase-3 Colorimetric Assay kit (TBS2030)
 Mitochondria Isolation (TBS2116)
 Mitochondria Complex 1 Activity Assay (TBS2017)
 Mitochondria Oxidase Activity (TBS2105)
 Mitochondrial Membrane Potential Assay (TBS2049)
 Cytochrome C Oxidase Activity Assay (TBS2115)

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