

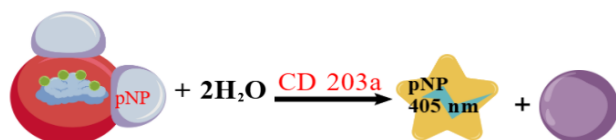
CD203a Activity Assay (TBS2113, 100 Assays, Store at -20°C)

DESCRIPTION

CD203a (ENPP1) is a key well-known cell surface marker and enzyme which hydrolyzes ATP, cGAMP and other substrates. It plays roles in purinergic signaling, bone and soft tissue mineralization, immune regulation, cGAS/STING pathway modulation and tumor microenvironment regulation. It is very important in both basic research and clinical applications.

Tribioscience CD203a Colorimetric Assay provides a simple and sensitive method for monitoring CD203a activity in biological samples (cells, tissue, serum, urine, stool). This assay uses a synthetic CD203a substrate to release p-nitrophenol (pNP) in the presence of CD302a enzyme. The absorbance of pNP is measured at 405 nm as shown in Fig. 1.

Fig. 1: Assay Principle



Substrate

Synonyms: ENPP1; PC-1; ARHR2; COLED; M6S1; PDNP1

APPLICATIONS

Determination of CD203a activity in biological samples.

KEY FEATURES

Flexible: can be used for 96 wells and 384 wells plate.

Simple: Just one-step: add-incubate-read model.

Time saving: a 30-minute reaction at 37°C

KIT CONTENTS

Component	100x Rxns
CD203a Substrate (20x)	0.45 mL
pNP Standard (10mM)	0.1 mL
Positive control (10x)	5 µL
Assay Buffer	12 ml
Activator buffer	5 ml

STORAGE CONDITIONS

The kit is shipped on ice and should be stored at -20°C for shelf life of 12 months after receipt.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and assay buffer into samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation: Serum and plasma can be assayed directly. For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate

cells in an appropriate volume of cold PBS, approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

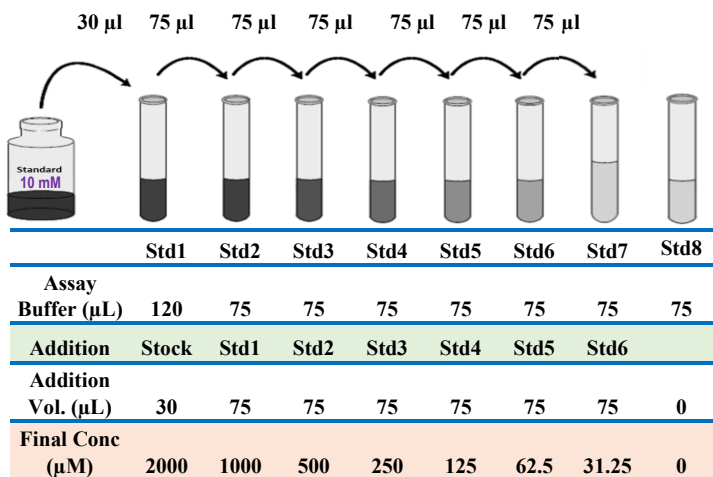
Reagent Preparation:

- Equilibrate all components to the room temperature. If substrate has precipitation in bottle, please warm up to dissolve the precipitation.
- Briefly vortex or pipette up and down all components (positive control can't be vortex) to ensure fresh reconstitution.

Reaction Preparation:

1. Label tubes as #1 through #8 as below table 1.
2. Add 120 µL of Assay Buffer to Std1, and 75 µL to Std2 to 8.
3. Pipet 30 µL of 10 mM standard stock into Std#1. Then, make 2x series dilution in Std2 through 7 with addition of 75 µL. Std8 is 1x Assay Buffer alone as a **standard 0**. The standard concentration in tube 1 through 7 will be 2000, 1000, 500, 250, 125, 62.5, 31.25µM, **Tube#8 is Standard 0 as blank**.

Fig.2: Diagram for CD203a standard preparation



4. Add 45 ul of assay buffer to the 10x positive control.
5. Dilute substrate stock (20x) with assay buffer for 10 times. For 96 well plate: 7.6 mL Assay buffer + 0.4 mL substrate stock (20x). Mix well gently.
6. Transfer 20 µL of each sample, blank, positive control, and standards into two separate wells.
7. Add 80 µL of the substrate solution to all samples, positive control, and blank wells. Add 80 µL of Assay Buffer to each standard wells (Note: Do not add substrate in the standard). Tap plate briefly to mix.
8. Incubate at 37°C for 30-60 minutes.
9. Read plate at OD 405nm in the endpoint mode.

CALCULATION

Subtract blank OD (Standard 0, #8) from the standard OD values and plot the ΔOD against standard concentrations. Determine the slope, and use the following equation to calculate CD203a activity:

$$\text{CD203a Activity (U/L)} = \text{DF} * (\text{ODSAMPLE} - \text{OD BLANK}) / (t * \text{Slope})$$

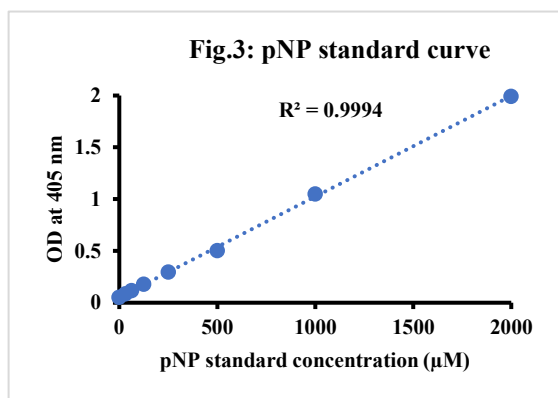
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where ODSAMPLE is the OD405nm value for each sample and ODBLANK is the OD405nm value of the sample blank. Slope is the linear regression fit of the standard points and t is the reaction time (30 min). DF is the dilution factor.

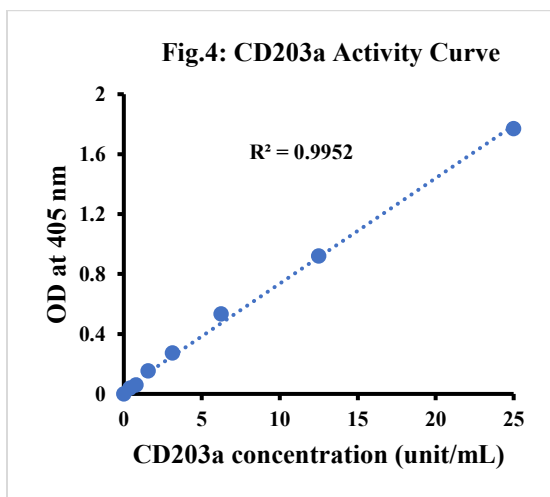
Unit definition: 1 Unit (U) will catalyze the conversion of 1 μ mole of substrate to p-Nitrophenol per min at 37°C.

TYPICAL DATA

This standard curve is provided for demonstration only on **Fig.3**. A standard curve should be generated for each set of samples assayed.



TYPICAL DATA: Different concentrations of enzyme with substrate shown as Fig. 4.



RELATED PRODUCTS

CD38 Cyclase Activity Assay (TBS2100)
 CD38 Hydrolase Activity Assay (TBS2047)
 CD73 Activity Assay (TBS2046)
 CD26 Activity Assay (TBS2210)
 CD26 Inhibitor Screening (TBS2104)
 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)
 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)

Research use only.