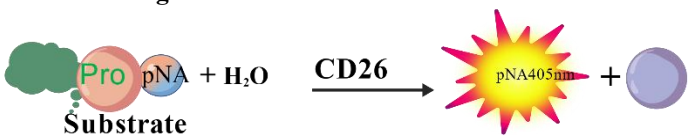
Tribioscience

CD26 (DPP4) Activity Colorimetric Assay Catalog: TBS2210
(100 Assays, Store at -20°C)

DESCRIPTION

CD 26 (dipeptidyl peptidase-4/DPP4) is a transmembrane glycoprotein expressed on many cell types, including immune cells, epithelial cells, and endothelial cells. Its enzymatic activity: cleaves dipeptides from the N-terminus of polypeptides when the second amino acid is proline or alanine. It is used to manage and reduce inflammation and is related to some diseases, such as Alzheimer's disease, diabetes, and cancer.

Tribioscience CD26 Activity Colorimetric Assay provides a simple and sensitive method for monitoring CD26 activity in biological samples (tissue, cells, serum, urine, stool). This assay uses a synthetic p-nitroaniline derivative (R-*p*NA) as its substrate. CD26 can specifically cleave the substrate to release *p*NA, a chromophore which can be measured at absorbance (OD 405 nm) as shown in **Fig. 1.**



Synonyms: TP103; Dipeptidyl peptidase IV; ADABP/ADCP2.

APPLICATIONS

This kit is used for the determination of CD26 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
CD26 Substrate (50x)	0.18 mL
pNA Standard (10 mM)	0.1 mL
Positive control (10x)	5 µL
Assay Buffer	12 ml

STORAGE CONDITIONS

The kit is shipped on ice and should be stored at -20°C for long-term storage. 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 to 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.

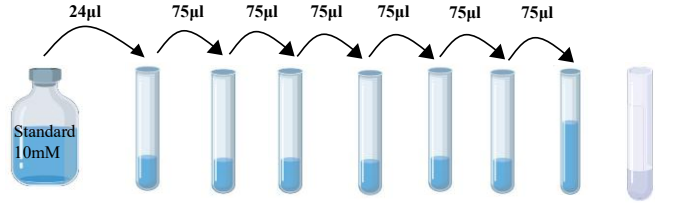
Reagent Preparation:

- 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 table1.
2. Add 126 µL of Assay Buffer to Std1, and 75 µL to Std2 to 8.
3. Pipet 24 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 1600, 800, 400, 200, 100, 50, 25µM, Tube#8 is Standard 0 as blank.

Table 1:



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	126	75	75	75	75	75	75	75
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	24	75	75	75	75	75	75	0
Final Conc (µM)	1600	800	400	200	100	50	25	0

4. Add 45 ul of assay buffer to the 10x positive control.
5. Dilute substrate stock (50x) with assay buffer for 50 times. For 100 well plate: 7.84 mL Assay buffer + 0.16 mL substrate stock (50x). 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 CD26 activity:

CD26 Activity (U/L) = DF * (ODSAMPLE – OD BLANK)/ (t * Slope)

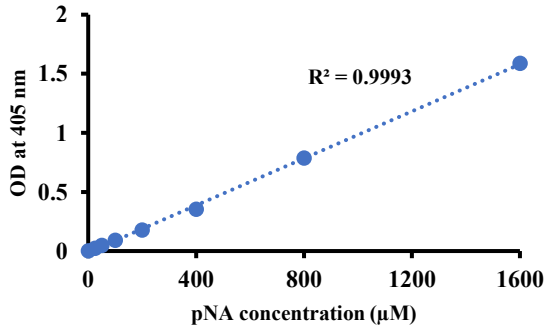
where ODSAMPLE is the OD405nm value for each sample and ODBLANK is the OD405nm value of the sample blank. Slope is the slope of the linear regression fit of the standard points and t is the reaction time (30 min or 60 min). DF is the dilution factor.

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

TYPICAL DATA

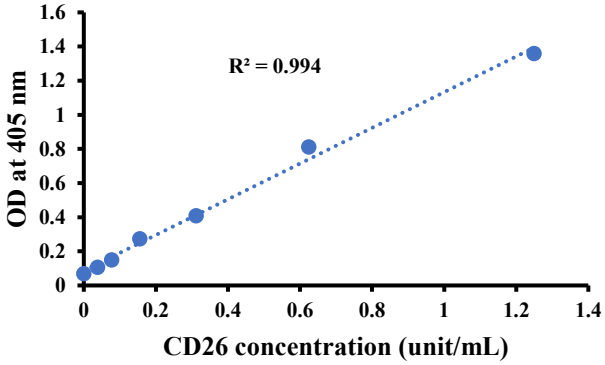
This standard curve is provided for demonstration only as below **Fig.2.** A standard curve should be generated for each set of samples assayed.

Fig. 2: pNA standard



Typical data of different concentrations of enzyme with substrate shown as **Fig. 3.**

Fig.3: CD26 activity assay



RELATED PRODUCTS:

Caspase-3 Fluorometric Assay kit (TBS3230)
Tryptase Activity Assay (TBS2101)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

Research use only.