

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Calcineurin (CaN) Activity Assay Kit

Catalog No.: BC00150

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Calcineurin (CaN) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer or Microplate reader (optimal detection wavelength 636 nm)

Product Introduction

Calmodulin is a Ca^{2+} /calmodulin-dependent serine/threonine protein phosphatase that plays a key regulatory role in physiological and pathological processes such as T cell activation, neurotransmitter release, myocardial hypertrophy, and calcium signal transduction.

Principle

Calcineurin catalyzes the dephosphorylation of phosphorylated substrates to release free phosphate ions, which react with ammonium molybdate under acidic conditions to form ammonium phosphomolybdate, which is then reduced to molybdenum blue. Enzyme activity is quantified colorimetrically at 636 nm.

Components

Components	Name	Size	Storage
Reagent 1	Buffer solution	3 mL	2~8°C
Reagent 2	Powder	1 tube of powder	2~8°C
	Diluent	1.5 mL	2~8°C

Preparation of reagent 2 application solution: Add the powder to the diluent and dissolve.			
Reagent 3	Stock solution	0.015 mL	-20°C
	Diluent	0.15 mL	2~8°C
Preparation of Reagent 3 application solution: Prepare according to the ratio of Reagent 3 stock solution to Reagent 3 dilution solution = 1:10. Prepare only the amount needed for each use, and store the remainder below 20°C for 3 days.			
Reagent 4	Terminator	0.6 mL	2~8°C
Reagent 5	Acid	7 mL × 2	2~8°C
	Liquid B	6 mL × 2	2~8°C
Preparation of the colorimetric reagent: When using, add one bottle of reagent 5 Acid solution to one bottle of reagent 5 Liquid B solution (Liquid B may produce flocculent matter at low temperatures, requiring dissolution by heating in a 60°C water bath for 10 minutes before use), mix thoroughly, and prepare 0.5 hours in advance. It can be stored at 4°C for at least 5 days. If your sample volume is small, you can prepare only the required amount using a reagent 5 Acid solution: reagent 5 Liquid B solution ratio of 7:6. (When preparing the colorimetric reagent according to the ratio, prevent phosphorus contamination; it is best to use new disposable pipette tips or dedicated pipette tips).			
Reagent 6	Stabilizer	30 mL	2~8°C
Reagent 7	10 mmol/L standard phosphorus stock solution	1 mL	2~8°C
Preparation of 0.1 mmol/L standard phosphorus working solution: Dilute Reagent 7 standard phosphorus stock solution 100 times before use, i.e., take 0.1 mL of standard phosphorus stock solution and add double-distilled water to 10 mL. Prepare fresh before use.			
Consumables 1	Microplate (96 wells)	1 plate	RT
Consumables 2	Plate Sealer	2 pieces	RT

Storage

The kit has a shelf life of 3 months.

Operation process

Enzymatic reaction

Reagents(μ L)	Control tube	Test tube
Reagent-Buffer	60	60
Reagent 2 Substrate Solution	25	25
Reagent 3 Application Solution	3	3
Sample to be tested	--	25
Mix well and incubate in a 37°C water bath for 20 minutes.		
Reagent 4 Terminator	--	--
Sample	--	--
Mix well, centrifuge at 3500 rpm for 10 minutes, and take 50 μ L of the supernatant for phosphorus determination.		

Phosphorus reaction

Reagents(μ L)	Blank tube	Standard tube	Test tube	Control tube
Double-distilled water	50	--	--	--
0.1 mmol/L standard phosphorus solution	--	50	--	--
Test tube supernatant	--	--	50	--
Control tube supernatant	--	--	--	50
Reagent 5 colorimetric solution	500	500	500	500
Mix well and let stand for 2 minutes.				
Reagent 6 Stabilizer	500	500	500	500
Mix well, let stand at room temperature for 5 minutes, and measure the absorbance (OD) of each tube at 636 nm with a light path of 0.5 cm using double-distilled water to zero the light.				

Result calculation

Unit definition: One CaN activity unit is defined as the amount of inorganic phosphorus produced by calcineurin (CaN) per milligram of protein per hour by breaking down the substrate PNPP.

$$\text{CaN Activity (U/mgprot)} = \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times N \times \frac{60}{T} \div C_{\text{pr}}$$

Annotation

C_{standard} : Standard solution concentration, 0.1 mmol/L ($\mu\text{mol/mL}$).

N: Dilution factor of the reaction system, 5 (125 μL /25 μL);

T: Enzyme reaction time, 20 min.

C_{pr} : Protein concentration in tissue homogenate , mgprot /L (prot refers to protein) .

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.