

CaM-kinase II (Human) Assay Kit

Product Information

Cat.No.

Kit-0146

Product Overview

CaM-kinase II (Human) Assay Kit is a single-site, non-quantitative immunoassay for CaM kinase II activity. Plates are pre-coated with a newly designed Syntide-2, which can be efficiently phosphorylated by CaM kinase II on a microtiter plate. The detector antibody is MS-6E6, an antibody that specifically detects only the phosphorylated Syntide-2.

Description

Ca²⁺/CaM-dependent protein kinase II (CaM kinase II) is a ubiquitously expressed protein kinase that transduces elevated Ca²⁺ signals in cells to a number of target proteins ranging from ion channels to transcriptional activators. CaM kinase II has a unique holoenzyme structure and autoregulatory properties that allow it to give a prolonged response to transient Ca²⁺ signals and to sense cellular Ca²⁺ oscillations. In neurons CaM kinase II is highly expressed and localized with certain subcellular structures. Upon activation it can translocate to excitatory synapses where it regulates a number of proteins involved in synaptic transmission and its downstream signaling pathways. Changes in intracellular calcium can display variable responses ranging from highly localized, transient elevations within subcellular structures (e.g. a dendritic spine of a neuron) to Ca²⁺ waves that spread throughout the cell including the nucleus. The most ubiquitous calcium sensing protein is Calmodulin (CaM), which contains four "EF" hand motifs with high specificity for binding Ca²⁺. The Ca²⁺/CaM complex interacts with and modulates the functionality of a large number of proteins including several Ser/Thr protein kinases.

Applications

- 1) Monitoring the purification of CaM kinase II.
- 2) Screening inhibitors or activators of CaM kinase II.
- 3) Detecting the effects of pharmacological agents on CaM kinase II activity.

Target Species

Human

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Usage

For research use only (RUO)

Storage

- Upon receipt store the ATP at -20°C
- Upon receipt store all other components at 4°C; Do not expose reagents to excessive light

Kit Components

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with Syntide-2 as a substrate.10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2%Tween -20Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution.50X CaCl₂: One vial containing 0.4 mL of 125 mM CaCl₂, used for Kinase Reaction Buffer (Ca/CaM plus).50X EGTA: One vial containing 0.4 mL of 100 mM EGTA, used for Kinase Reaction Buffer (Ca/CaM minus).20X ATP: Lyophilized ATP Na₂ salt. Reconstitute contents of vial with 1.6 mL of H₂O. Mix gently until dissolved. Final concentration of ATP should be 1.25 mM ATP. The ATP solution can be stored in small aliquots (e.g. 100 µL) at -20°C. The 1.25 mM ATP stock solution must be diluted to 62.5 µM in Kinase Reaction Buffer (Ca/CaM plus) or Reaction Buffer (Ca/CaM minus) at the time of the assay. HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-phospho-Syntide-2 (MS-6E6) monoclonal antibody. Ready to use. Substrate Reagent: 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use. Stop Solution: One bottle supplied ready to use, containing 20 mL of 1 N H₂SO₄. Ready to use.