

## 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**

Ca<sup>2+</sup>/CaM-dependent protein kinase II (CaM kinase II) is a ubiquitously expressed protein kinase that transduces elevated Ca<sup>2+</sup> 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 Ca<sup>2+</sup> signals and to sense cellular Ca<sup>2+</sup> 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 Ca<sup>2+</sup> 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 Ca<sup>2+</sup>. The Ca<sup>2+</sup>/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

## CaM-kinase II (Human) Assay Kit

### 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-20. Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution. 50X CaCl<sub>2</sub>: One vial containing 0.4 mL of 125 mM CaCl<sub>2</sub>, 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 Na<sub>2</sub> salt. Reconstitute contents of vial with 1.6 mL of H<sub>2</sub>O. 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 H<sub>2</sub>SO<sub>4</sub>. Ready to use.