protein kinase MEK2 or MAP Kinase Kinase (MAPKKK) (1–4).

**Recognition Determinants:** The minimal recognition motif for phosphorylation by MAPK is S/TP. Pro is also common at the -2 position in the optimal primary motif PXS/TP. The substrate specificity of MAPK overlaps with other proline-directed protein kinases present within the cell. This suggests that the recognition of protein substrates may require structural determinants in addition to primary sequence requirements (5).

**Source:** Isolated from a strain of E. coli that carries a clone expressing murine MAPK (1) under the control of a T7 expression system. Fully active MAPK is produced by co-expression with a constitutively active form of its activator, MEK2 (6).

**Supplied in:** 100 mM NaCl, 50 mM HEPES (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.01% Brij 35 and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 10X NEBuffer for Protein Kinases (PK).
- 1X NEBuffer for PK (NEB #B6022), supplemented with 200 µM ATP (NEB #P0756) and gamma-labeled ATP to a final specific activity of 100–500 µCi/µmol. **Incubate at 30°C.**
- 1X NEBuffer for PK: 50 mM Tris-HCl 10 mM MgCl₂ 0.1 mM EDTA 2 mM DTT 0.01% Brij 35 pH 7.5 @ 25°C
- Note that optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

**Unit Definition:** One unit is defined as the amount of MAPK required to catalyze the transfer of 1 pmol of phosphate to myelin basic protein (50 µM) in 1 minute at 30°C in a total reaction volume of 30 µl.

**Specific Activity:** ~10,000,000 units/mg.

**Molecular Weight:** 42 kDa.

**Purity:** MAPK has been purified to > 95% homogeneity as determined by SDS-PAGE and Coomassie Blue staining.

**Quality Assurance:** MAPK contains no detectable protease, phosphatase or MEK Kinase activities.

**Quality Control Assays**

**Protease Activity:** After incubation of 500 units of MAP Kinase (MAPK) with a standard mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE analysis.

**Phosphatase Activity:** After incubation of 500 units of MAP Kinase (MAPK) with 50 mM p-Nitrophenylphosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

**MEK Kinase Activity:** After incubation of 500 units of MAP Kinase (MAPK) using unphosphorylated MAP Kinase as a substrate for 30 minutes at 30°C, no MEK Kinase activity could be detected by the phosphocellulose paper binding method.
Heat Inactivation: 65°C for 20 minutes.

Notes On Use: Avoid freeze/thaw cycles. Can be stored for 2 weeks or less at –20°C.

References:

U.S. Patent No. 5,763,244
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