p42 MAP Kinase (MAPK)

1-800-632-7799
info@neb.com
www.neb.com

P6080S 009130614061

P6080S

2,000 units **100,000 U/ml** **Lot: 0091306**

RECOMBINANT **Store at –70°C** **Exp: 6/14**

Description: p42 MAP Kinase (Mitogen-Activated Protein Kinase, MAPK), also known as Erk2 (Extracellular signal-regulated kinase 2) is one of two isoforms of MAP kinase family. It is a serine/threonine protein kinase, participating in mammalian signal transduction pathways that control intracellular responses to hormones and major developmental changes. Full activation of p42 MAP Kinase requires phosphorylation at residues T183 and Y185 catalyzed by the upstream protein kinase MEK2 or MAP Kinase Kinase (MAPKK) (1–4).

Recognition Determinants: The minimal recognition motif for phosphorylation by MAPK is **S/T**P. Pro is also common at the -2 position in the optimal primary motif PX**S/T**P. 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).

Reaction Conditions: 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 $MgCl_2$
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.

(see other side)

CERTIFICATE OF ANALYSIS

p42 MAP Kinase (MAPK)

1-800-632-7799
info@neb.com
www.neb.com

P6080S 009130614061

P6080S

2,000 units **100,000 U/ml** **Lot: 0091306**

RECOMBINANT **Store at –70°C** **Exp: 6/14**

Description: p42 MAP Kinase (Mitogen-Activated Protein Kinase, MAPK), also known as Erk2 (Extracellular signal-regulated kinase 2) is one of two isoforms of MAP kinase family. It is a serine/threonine protein kinase, participating in mammalian signal transduction pathways that control intracellular responses to hormones and major developmental changes. Full activation of p42 MAP Kinase requires phosphorylation at residues T183 and Y185 catalyzed by the upstream protein kinase MEK2 or MAP Kinase Kinase (MAPKK) (1–4).

Recognition Determinants: The minimal recognition motif for phosphorylation by MAPK is **S/T**P. Pro is also common at the -2 position in the optimal primary motif PX**S/T**P. 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).

Reaction Conditions: 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 $MgCl_2$
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.

(see other side)

CERTIFICATE OF ANALYSIS

References:

1. Boulton, T.G. et al. (1991) *Cell* 65, 663–675.
2. Rossomando, A.J. et al. (1991) *J. Biol. Chem.* 266, 20270–20275.
3. Payne, D.M. et al. (1991) *The EMBO Journal* 10, 885–892.
4. Prowse, C.N. et al. (2001) *J. Biol. Chem.* 276, 40817–40823.
5. Davis, R.J. et al. (1993) *J. Biol. Chem.* 268, 14553–14556.
6. Wu, J. et al. (1993) *Mol. Cell Biol.* 13, 4539–4548.

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

References:

1. Boulton, T.G. et al. (1991) *Cell* 65, 663–675.
2. Rossomando, A.J. et al. (1991) *J. Biol. Chem.* 266, 20270–20275.
3. Payne, D.M. et al. (1991) *The EMBO Journal* 10, 885–892.
4. Prowse, C.N. et al. (2001) *J. Biol. Chem.* 276, 40817–40823.
5. Davis, R.J. et al. (1993) *J. Biol. Chem.* 268, 14553–14556.
6. Wu, J. et al. (1993) *Mol. Cell Biol.* 13, 4539–4548.

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

Page 2 (P6080)