

Product Information

Anti-phospho-ERK5 (pThr²¹⁸/pTyr²²⁰)

produced in rabbit, affinity isolated antibody

Product Number **E7153**

Product Description

Anti-phospho-ERK5 (BMK1) (pThr²¹⁸/pTyr²²⁰) is produced in rabbit using a synthetic phosphopeptide derived from the region of human ERK5 (BMK1) containing threonine 218 and tyrosine 220 as immunogen. The sequence is conserved in human and mouse. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards a non-phosphorylated ERK5 enzyme and towards phosphorylated ERK1 and ERK2 enzymes.

Anti-phospho-ERK5 (BMK1) (pThr²¹⁸/pTyr²²⁰) specifically detects human ERK5 (88 kDa). Mouse ERK5 (100% homologous) has not been tested, but is expected to react. Rat has also not been tested. It has been used in immunoblotting^{1,2} and immunostaining.

Extracellular Signal-Regulated Kinases (ERKs) are members of the mitogen-activated protein kinase superfamily (MAPK). MAPK cascade is an evolutionary conserved module that mediates the signaling from various extracellular stimuli to the nucleus. The core elements of a MAPK pathway are three sequentially activated protein kinases, terminating in MAP kinase family members. In mammals a MAP/ERK kinase (MEKK) activates a MAP/ERK kinase (MEK), which activates an ERK or MAP kinase.

There are three well defined mammalian MAP kinase modules: the ERK1/2 module, the c-Jun N-terminal protein kinase/stress-activated protein kinase module, and the p38 module. ERK3, ERK4, and ERK5 and other p38 isoforms have also been identified, but the cascades leading to activation of these kinases are not well characterized. Each member of MAPKs is activated by both tyrosine and threonine phosphorylation, catalyzed by a distinct upstream kinase, a member of the MAPK kinase family.^{3,4,5,6}

The well-characterized ERK module is activated in response to stimuli such as cytokines, growth factors, osmotic shock or UV irradiation. ERKs regulate transcription, cell cycle, differentiation, learning and memory through signal transduction in the cytoplasm

and the nucleus. ERK1 and 2 phosphorylate microtubule-associated protein-2 (MAP2), myelin basic protein (MBP) and ELK-1. They may promote entry in the cell cycle. The ERK cascade connects to G proteins through a multitude of distinct signal transduction pathways. Both receptor and non-receptor tyrosine kinases play roles in these signaling pathways.⁷ ERK1 (p44) and ERK2 (p42) require the dual phosphorylation in the catalytic kinase domain by MEKs for their full activity. ERK1 is phosphorylated on Tyr²⁰⁴ and Thr²⁰², and ERK2 on Tyr¹⁸⁷ and Thr¹⁸⁵. ERK1 and 2 may also undergo autophosphorylation on these residues. Recent studies have found activated ERK1 and ERK2 in agonist-stimulated platelets, in core proteins of chronic hepatitis C (HCV) virus, and in angiogenin-activated umbilical vein endothelial cells.^{7,8,9} G proteins, NO, and ROS play an essential role in ERK1/2 activation.

Recently, ERK5 (also known as BMK1) was identified as a novel member of MAPK family. This kinase is strongly activated by oxidative and osmotic stresses, epidermal growth factor, and nerve growth factor, whose receptors are tyrosine kinases. ERK5 is activated via H-Ras and Src and is phosphorylated by c-Myc, which suggests a kinase cascade involved in normal and pathological cellular processes, such as cancer.^{11,12} The ERK5 is phosphorylated on Thr²¹⁸ and Tyr²²⁰. MEK5/ERK5 MAP kinase cascade is critical for early steps of muscle cell differentiation, the induction of special form of cardiac hypertrophy, and enhanced neuronal survival.

Reagent

Supplied as a solution in Dulbecco's phosphate buffered saline, pH 7.3, with 50% glycerol, 1 mg/mL BSA and ≤ 0.1% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at -20°C . For extended storage, upon initial thawing, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in a frost-free freezer. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

Direct Immunoblotting: each vial contains enough material for 10 blots.

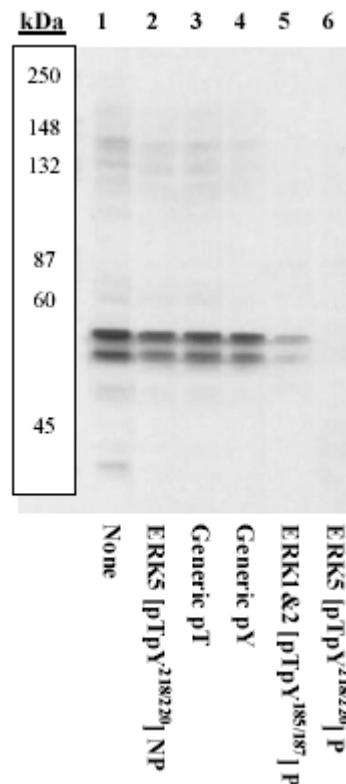
Due to the low levels of activation of endogenous ERK5, overexpression or immunoprecipitation of ERK5 protein may be required for detection. Some cross-reactivity with ERK1/ERK2 may be observed.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test

Peptide Competition

1. Extracts prepared from HEK293 cells transiently transfected with plasmids expressing ERK5 kinase domain (ERK5kin) and constitutively activated MEK5D-D were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4°C .
3. After blocking, membranes were preincubated with different peptides as follow:
Lane 1 no peptide
Lane 2 non phosphorylated peptide corresponding to the immunogen
Lane 3 a generic phosphothreonine containing peptide
Lane 4 a generic phosphotyrosine containing peptide
Lane 5 the phosphopeptide derived from the corresponding region of ERK1&2 immunogen
Lane 6 immunogen
4. After preincubation membranes were incubated with Anti-phospho-ERK5 (pThr²¹⁸/pTyr²²⁰) for two hours at room temperature in a 3% BSA TBST buffer,

5. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase conjugate and bands were detected using the Tropix[®] WesternStar[™] detection method.



The data show that while there is some cross-reactivity with ERK1&2, only the phosphopeptide corresponding to ERK5 (BMK1) (pThr²¹⁸/pTyr²²⁰) completely blocks the antibody signal, demonstrating the specificity of the antibody.

Note: The antibody signal appears at ~ 50 kDa as this is the molecular weight of the transiently transfected ERK5 kinase domain.

References

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