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ProductInformation

Anti-phospho-MLK3 [pThr^{277}/pSer^{281}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 6318**

Product Description

Anti-phospho-MLK3 (Member of the mixed-lineage kinase family) [pThr^{277}/pSer^{281}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human MLK3 that contains threonine 277 and serine 281as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated MLK3 peptide. Antibody specifically recognizes MLK3 phosphorylated at threonine 277 and serine 281.

The antibody detects human MLK3. Mouse (100% homology) has not been tested. The antibody has been used in immunoblotting applications.

Members of the mixed-lineage kinase (MLK) family (including MLK1, MLK2, MLK3, and dual leucine zipper kinase [DLK]) are serine/threonine protein kinases that are expressed in multiple cell types. MLK3 is activated by phosphorylation in response to stress stimuli (e.g., inflammatory responses, UV, chemical stress) that are coupled to the small GTPase, Cdc42/rac. MLK3 is a multifunctional kinase that plays an essential role in several signaling pathways, including mitogen-activated protein kinase (i.e., activation of JNK and p38), IkappaB/NFkappaB, and p70 S6 kinase. Indeed MLK3 signaling occurs through multiple signaling domains in this protein kinase including (from N- to C-terminal) a glycine-rich domain, Src homology 3 (SH3) domain, a kinase domain, a zipper domain, a Cdc42/rac interactive binding (CRIB) domain and a Pro/Ser/Thr-rich domain.

Phosphorylation of MLK3 occurs on multiple residues including threonine 277 and serine 281 within the activation loop of the kinase domain.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

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Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at –70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 immunoblots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using 293 cell lysates transfected with wild-type or mutant MLK3.

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

Results

Western Blot

1. Extracts prepared from 293 cells were transfected with:
    a. the mutant T277A/S281A MLK3 (lanes 1, 5),
    b. single mutant S281A MLK3 (lanes 2, 6),
    c. single mutant T277A MLK3 (lanes 3, 7),
    d. or wild-type MLK3 (lanes 4, 8)
2. Extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel, and transferred to PVDF.
3. Mutant MLK3 protein extracts were tagged with Flag; wild-type MLK3 was tagged with HA.

4. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C, then were incubated for 2 hours at RT in a 3% BSA-TBST buffer, with:
    a. anti-Flag antibody (lanes 1-3),
    b. anti-HA antibody (lane 4),
    c. 0.75 µg/mL MLK3 [pThr^{277}/pSer^{281}] antibody (lanes 5-8)
5. After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and bands were detected.

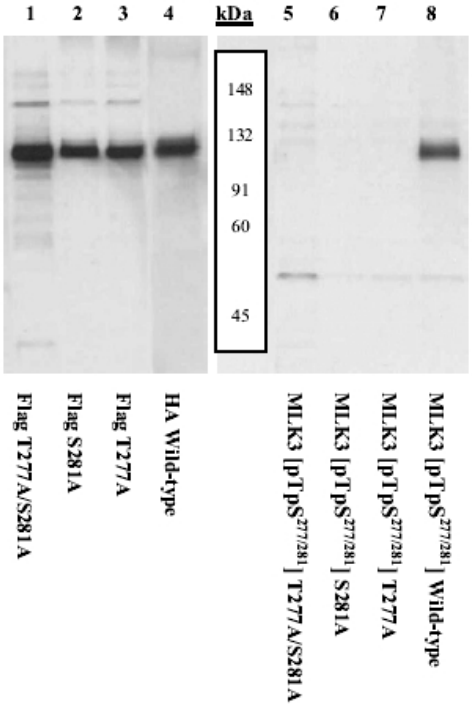

Figure 1 Western Blot

The data show that the MLK3 [pThr^{277}/pSer^{281}] antibody is only reactive with wild-type MLK3, demonstrating the specificity of the antibody.)

References

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