# ProductInformation

**ANTI-SKK5 (MEK5)**
Developed in Rabbit, Affinity Isolated Antibody

Product Number **S5433**

## Product Description

Anti-SKK5 (MEK5) is developed in rabbit using a synthetic peptide (AFEYEDEDGDRITVRSC) corresponding to amino acids 59-74 of human SKK5 conjugated to KLH as immunogen. Stress activating protein kinase kinase 5 (SAPKK5, SKK5) is also termed MAP kinase kinase 5 (MEK5, MKK5). The antibody is purified using affinity chromatography.

Anti-SKK5 reacts specifically with human SKK5/MEK5 (49 kD) by immunoblotting. It should not cross-react with MEK1, MEK2, MEK3 or MEK4 (SEK1). It also reacts with mouse and rat SKK5. Other species cross-reactivity is unknown.

Anti-SKK5 may be used for the detection of SKK5 by immunoprecipitation and immunoblotting of A431, PC-12, C6 and 3T3 cell lysates.

Stress Activating Protein Kinase Kinase 5 (SKK5), also termed MAP Kinase Kinase 5 (MEK5) is a 49 kD MAP kinase kinase that catalyzes tyrosine phosphorylation of ERK5.[1] in some cell types the MEK5/BMK1 MAP kinase signaling pathway regulates serum-induced early gene expression through the transcription factor MEF2C.[2]
Big MAP kinase (Bmk1), also termed Erk5, is a member of the MAP kinase family that is activated in cells in response to oxidative stress, hyperosmolarity and treatment with serum. Epidermal Growth Factor (EGF) is a potent activator of Bmk1. In contrast to Erk1/2, EGF-mediated activation of Bmk1 occurs independently of Ras and requires the MAP-kinase kinase Mek5.[3]

## Reagents

The product is supplied as affinity isolated antibody in 0.07 M tris-glycine buffer, pH 7.0, containing 30% glycerol and 0.035% sodium azide (see MSDS)* as a preservative.

## 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 hazardous and safe handling practices.

**Storage/Stability**
Store at 0°C to –20°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

**Procedure**
*Immunoprecipitation*
1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 µg of anti-SKK5/MEK5 to 500 µg - 1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Sigma Product No. P2545).
5. Gently rock reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl of 2X Laemmli sample buffer, or the agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM $Na_3VO_4$, and 1 mM NaF.

**Product Profile**
Recommended working concentration is 1 µg/ml by immunoblotting using cell lysates from A431, PC-12, C6 and 3T3 cells.
Pre-washing the membrane with 0.05% Tween-20/PBS for 20-30 minutes prior to blocking the membrane is recommended to enhance immunoblotting.

For immunoprecipitation, 4 µg will immunoprecipitate SKK5 from 0.5 mg of a PC-12 cell lysate.

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

**References**

1. Zhou G, J Biol Chem, **26**, 12665 (1995).
2. Kato, Y., et al., EMBO J., **16**, 7054 (1997).
3. Kato, Y., et al., Nature, **395**, 713 (1998).


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