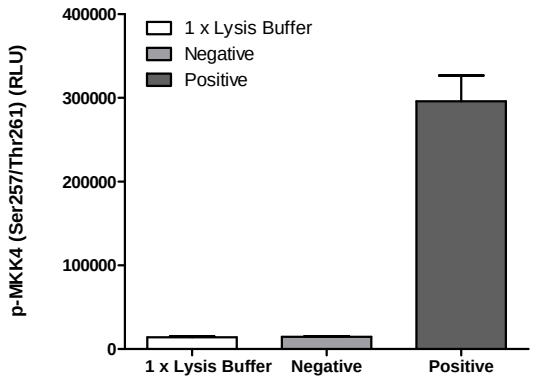
AlphaScreen® SureFire® phospho-MKK4 (Ser257/Thr261) Kit

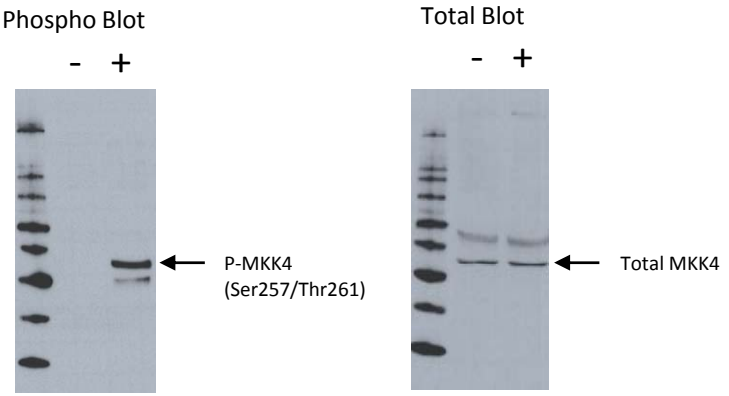
Catalog #
TGRMK4S500
TGRMK4S10K
TGRMK4S50K

MKK4 (also known as MEK-4 or SEK1) is a member of the MAPKK family of proteins, and activates the MAPK members JNK and p38 MAPK in response to cellular stresses and inflammatory cytokines. Activated MKK4 phosphorylates its substrates at the conserved Thr-Pro-Tyr sites in the activation loop of either p38 MAPK or JNK. MKK4 is itself activated by dual phosphorylation at Ser257/Thr261, by several upstream kinases in response to cellular stress. MKK4 activity is also negatively regulated by Akt phosphorylation at Ser80.

A



B



Cell Handling Protocol:
2 x T175 Flasks of confluent HEK293 cells were treated with either 1M D-Sorbitol for 45 minutes at 37°C (+ve), or left untreated (-ve).

Assay Protocol:
The media was removed and the cells were lysed in 5 mL/flask of 1x Lysis buffer with shaking for 10 minutes.
The lysates were analyzed for p-MKK4 (Ser257/Thr261) using either **(A)** the standard 2-plate AlphaScreen® *SureFire*® protocol for p-MKK4 or **(B)** by Western Blot (15µL lysate/lane) using the same antibodies used in the AlphaScreen® *SureFire*® Reaction buffer.

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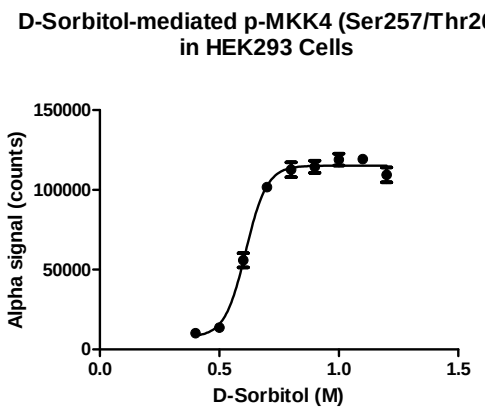


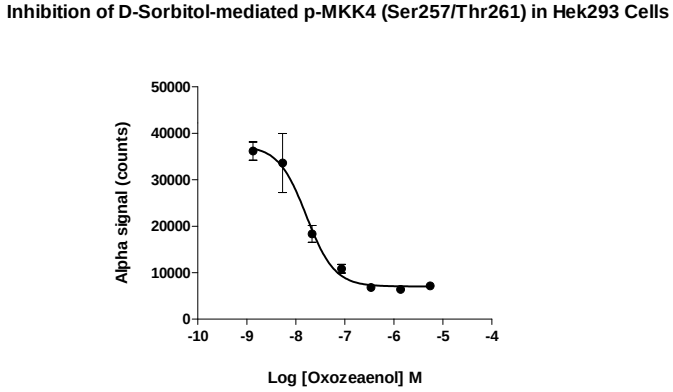
Agonist Dose Response Curve:

Cell Handling Protocol:
HEK293 cells were seeded at a density of 300K/mL (60K cells/well), in media containing 10% FBS. Cells were incubated overnight at 37°C.

Assay Protocol:
The following day the media was removed and the cells were dose stimulated with various concentrations of D-Sorbitol for 45 minutes at 37°C. The media was removed from the wells and the cells were lysed with 50µL/well 1X Lysis buffer with shaking for 10 minutes.

A portion of lysate (4µL) were transferred to a 384-well Proxiplate and analyzed for phospho-MKK4 (Ser257/Thr261) using the standard 2-plate AlphaScreen® *SureFire*® Protocol.

B



Inhibitor Dose Response Curve:

Cell Handling Protocol:
HEK293 cells were seeded at a density of 250K/mL (50K cells/well), in media containing 10% FBS. Cells were incubated overnight at 37°C.

Assay Protocol:
The following day the media was removed, and the cells were treated with various concentrations of Oxozeaenol for 30 minutes at 37°C, then stimulated with D-Sorbitol (1M) for 45 minutes at 37°C. The media was removed from the wells and the cells were lysed with 50µL/well 1X Lysis buffer with shaking for 10 minutes.

A portion of lysate (4µL) were transferred to a 384-well Proxiplate and analyzed for phospho-MKK4 (Ser257/Thr261) using the standard 2-plate AlphaScreen® *SureFire*® Protocol.