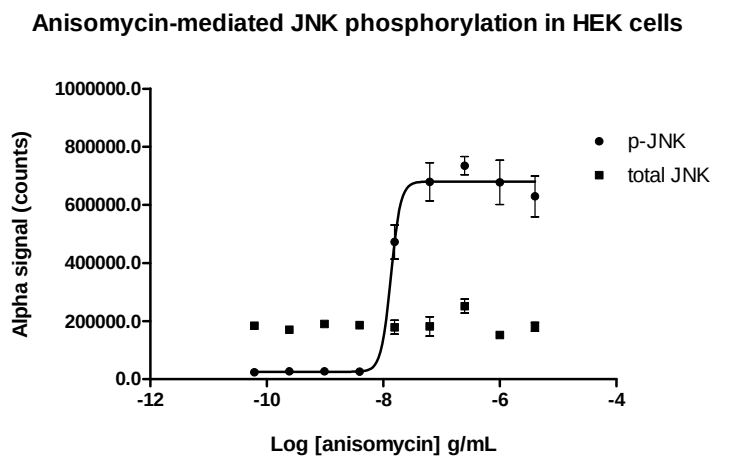
AlphaScreen® SureFire® phospho-JNK1/2/3 (Thr183/Tyr185) Kit

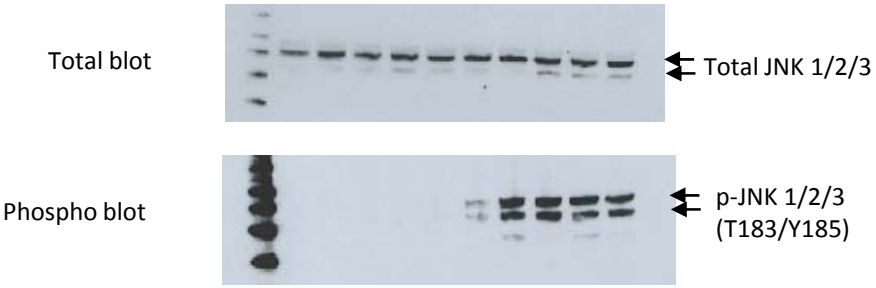
Catalog #
TGRJPS500
TGRJPS10K
TGRJPS50K

c-Jun N-terminal kinases (JNKs), are mitogen-activated protein kinases which are activate in response to stress stimuli, including inflammatory cytokines, ultraviolet irradiation, heat shock, and osmotic shock. JNK proteins are encoded by three genes, JNK1, JNK2 and JNK3, which undergo alternate splicing to generate several isoforms. JNK proteins are activated by phosphorylation, which is mediated through a classical MAPKKK/MAPKK/MAPK cascade, which in turn can be initiated through several mechanisms, generally in response to cellular stress signals. Activated JNKs can phosphorylate several proteins, including the transcription factor c-Jun.

A



B



Cell handling Protocol:
Hek 293 cells were seeded at 150k/mL (200µL/well giving 30K cells/well) in a 96 well plate in media containing 10% FBS. Cells were grown overnight at 37°C.

Assay Protocol:
The following day the media was removed and the cells were stimulated with various concentrations of **Anisomycin** for 30 minutes at 37°C.

The media was removed from the cells and the cells were lysed with 50µL/well of freshly prepared 1 x Lysis buffer with agitation for 10 minutes.

Lysates were then analysed for Phospho JNK 1/2/3 (Thr183/Tyr185) and Total JNK 1/2/3 using either **(A)** the standard 2-plate AlphaScreen® *SureFire*® protocol (4µL lysate per 384 Proxiplate well) or **(B)** Western blot (11.25µL lysate/lane) using the same antibodies used in the *SureFire*® Reaction buffer.

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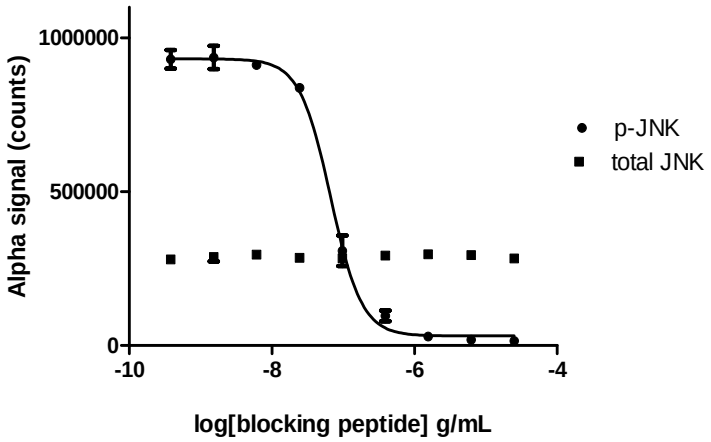
Inhibition of phospo-specific signal with JNK phospho-peptide



Cell Handling Protocol:
A T175 Flask of confluent HEK cells was treated with **Anisomycin** (1µg/ml) for 30 minutes at 37°C The media was removed and the cells were lysed in 5mL of 1x Lysis Buffer with agitation.

Assay Protocol:
Phospho-SAPK/JNK (Thr183/Tyr185) blocking peptide (Cell Signaling Technology, Cat#1215) was titrated into the lysate at various concentrations. Lysate containing the phospho-peptide was transferred to a 384-well Proxiplate, and analyzed for either p-JNK 1/2/3 (Thr183/Tyr185) or total JNK 1/2/3 using the standard 2-plate AlphaScreen® *SureFire* ® protocol.

The phospho-peptide in the lysates effectively blocked the phospho-dependant signal in the phospho-JNK assay, but had no effect on the total JNK detected in the lysate.

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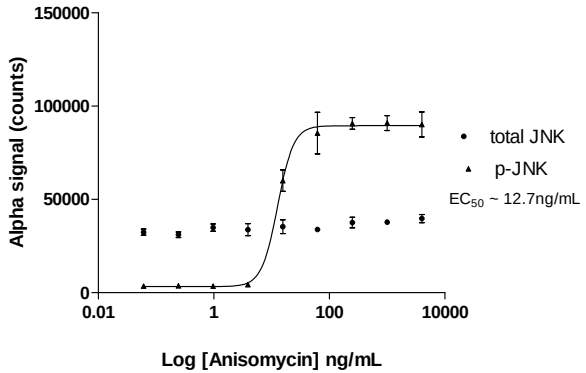
Anisomycin-mediated JNK phosphorylation in HEK Cells



Agonist Dose Response Curve:

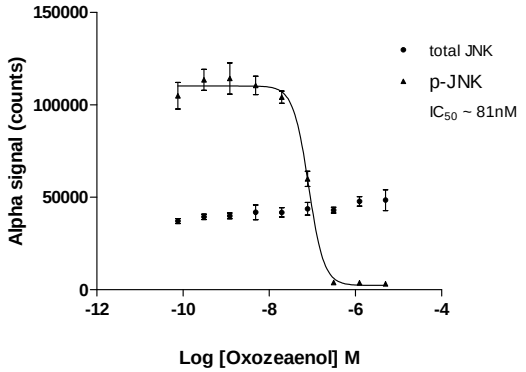
Cell Handling Protocol:
Hek Cells were seeded at 150K/mL in a 96 well plate (200µL/well giving 30K cells/well) in media containing 10% FBS. Cells were incubated overnight at 37°C.

Assay Protocol:
The following day the media was removed and the cells were dose stimulated with various concentrations of **Anisomycin** for 30 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.

Lysates (4µL) were then transferred to replicate wells in a 384-well Proxiplate and analysed for either total and phospho JNK 1/2/3 (T183/Y185) using the standard 2-plate AlphaScreen® *SureFire*® protocol.

Inhibition of Sorbitol-mediated JNK phosphorylation in Hek Cells



Inhibitor Dose Response Curve:

Cell Handling Protocol:
Hek Cells were seeded at 150K/mL in a 96 well plate (200µL/well giving 30K 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 inhibited with various concentrations of **Oxozeaenol** for 60 minutes at 37°C, then stimulated with **D-Sorbitol** (0.5M) for 30 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.

Lysates (4µL) were then transferred to replicate wells in a 384-well Proxiplate and analysed for either total and phospho JNK 1/2/3 (T183/Y185) using the standard 2-plate AlphaScreen® *SureFire*® protocol.