

AlphaScreen® SureFire® Total-JNK1/2/3 Assay Kit

Catalog #

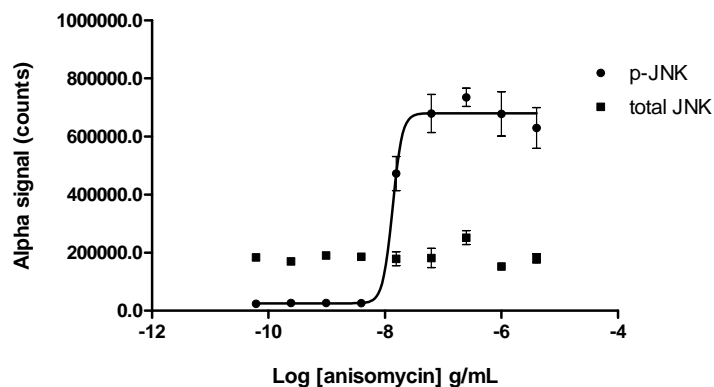
TGRTJS500

TGRTJS10K

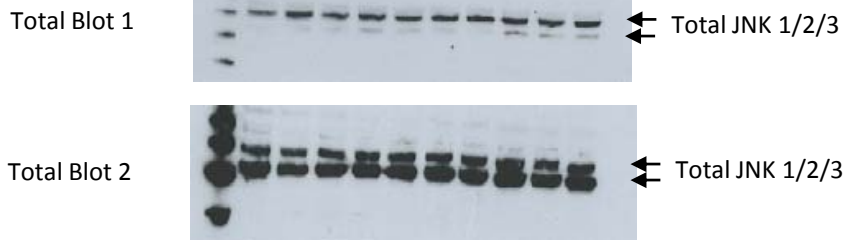
TGRTJS50K

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 Anisomycin-mediated JNK phosphorylation in HEK cells



B



Cell handling Protocol:

HeK 293 cells were seeded at 150k/mL (200 μ L/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 50 μ L/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 (4 μ L lysate per 384 Proxiplate well) or **(B)** Western blot (11.25 μ L lysate/lane) using the same antibodies used in the SureFire® Reaction buffer.

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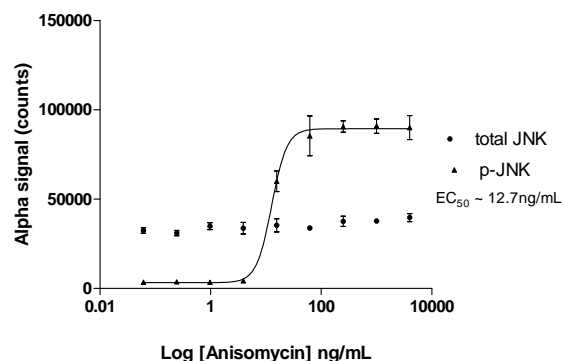
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TGRTJS500

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TGRTJS50K

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.

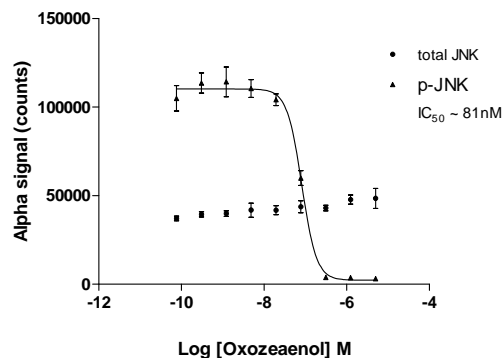
Assay Protocol:

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 **Oxozaenol** 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.