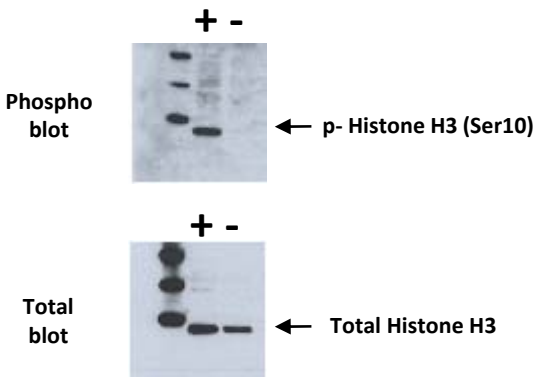
AlphaScreen® *SureFire*® phospho-Histone H3 (Ser10) Kit

Catalog#
TGRH3S500
TGRH3S10K
TGRH3S50K

Octameric histone complexes with DNA to form the nucleosome, the basic unit of chromatin. The nucleosome consists of approximately 200 base pairs of DNA, wound around a histone octamer comprised of 2 each of Histone H2A, H2B, H3 and H4. When chromatin is tightly packed, the binding of transcription factors is restricted. The N-terminus of histones undergo various types of modification, including acetylation, methylation, ubiquitinatin and phosphorylation. These modifications, which can be mediated by many different stimuli, modulate the accessibility of chromatin for transcription factors and subsequent gene expression. Histone H3, is phosphorylated at Ser10 and other N-terminal sites, and is correlated with chromatin condensation during mitosis and meiosis.



Cell Handling Protocol:
Confluent flasks of U2OS cells were treated with (+) nocodazole for 18 hours, or left untreated. The media was removed , and the cells were lysed with 1x Lysis buffer, with shaking for 10 minutes.

Assay Protocol:
The lysates were then analyzed for phospho-Histone H3 (Ser10) or total Histone by Western Blot (13.5µL lysate/lane) using the same antibodies used for the *SureFire*® Reaction Buffer.

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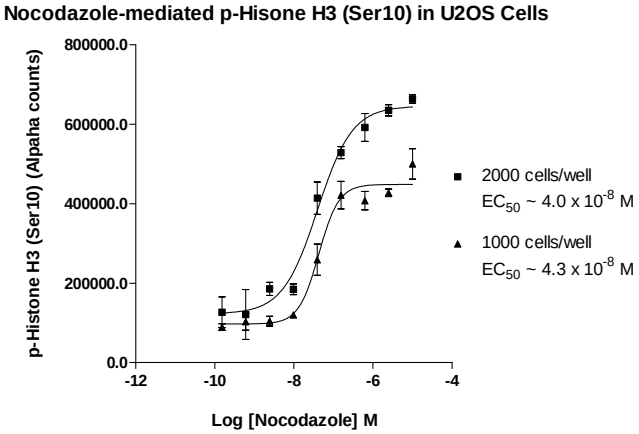
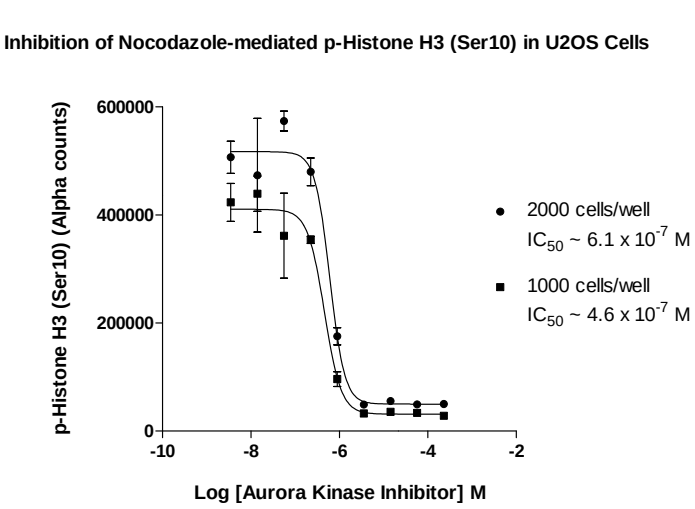


Agonist Dose Response Curve:

Cell Handling Protocol:
U2OS cells (Passage 39) were seeded at densities of 5K or 10K cells/ml (200µL/well giving 2K and 1K cells/well respectively) in a 96 well plate in media containing 10% FBS. Cells were incubated for 6 hours at 37°C.

Assay Protocol:
The media was removed from the wells, and the cells were stimulated with a range of concentrations of nocodazole diluted in media containing 10% FBS for 17 hours at 37°C. The media was then removed from the wells, and the cells were lysed with 100µL/well freshly prepared 1x Lysis buffer containing Activation buffer, with shaking for 10 minutes.

Lysates (5µL) were transferred to a 384-well Proxiplate™ and analyzed for phospho-Histone H3 (Ser10) using the standard 2-plate AlphaScreen® *SureFire*® protocol for Histone H3 detection.



Inhibitor Dose Response Curve:

Cell Handling Protocol:
U2OS cells (Passage 41) were seeded at densities of 5K or 10K cells/ml (200µL/well giving 2K and 1K cells/well respectively) in a 96 well plate in media containing 10% FBS. Cells were incubated for 6 hours at 37°C.

Assay Protocol:
The media was removed from the wells. and the cells were pre-incubated with a range of concentrations of Aurora Kinase Inhibitor (Calbiochem Cat#189406) for 30 minutes at 37°C, and then stimulated with nocodazole (10µM) for 16 hours at 37°C. The media was removed from the wells, and the cells were lysed with 100µL/well freshly prepared 1x Lysis buffer containing Activation buffer, with shaking for 10 minutes.

Lysates (5µL) were transferred to a 384-well Proxiplate and analyzed for phospho-Histone H3 (Ser10) using the standard 2-plate AlphaScreen® *SureFire*® protocol for Histone H3 detection.