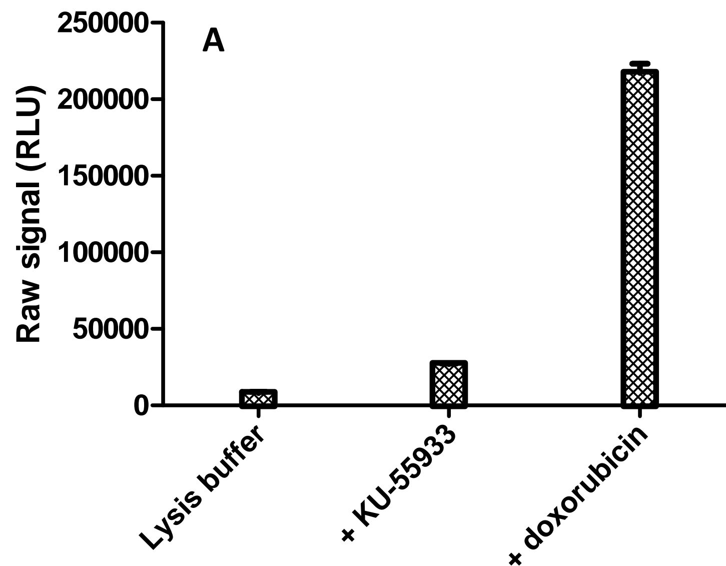
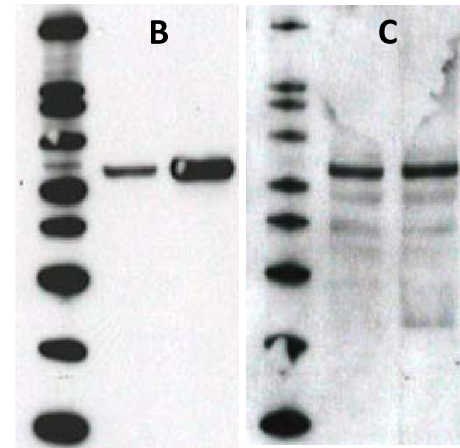
# AlphaScreen® *SureFire®* phospho-Chk2 (Thr68) Kit

| Catalog # |
|---|
| TGRCHK2S500 |
| TGRCHK2S10K |
| TGRCHK2S50K |

Chk1 and Chk2 are involved in the regulation of cell cycle events. They are involved in these processes as regulators of Cdks, and are essential components in the G2 DNA damage checkpoint, where they phosphorylate Cdc25C in response to DNA damage, which inhibits Cdc25C activity, and blocks mitosis. Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases. The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues. Following DNA damage induced by ionizing radiation, UV irradiation or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR kinases. This serine/threonine cluster, therefore, appears to have an important regulatory function. Phosphorylation at Thr68 is required for the subsequent kinase activation, via autophosphorylation of Chk2 on residues Thr383 and Thr387.




Lysates from HEK293 flasks stimulated with KU-55933 or doxorubicin for 60 minutes, were lysed were assayed for (A) phospho-Chk2 using the standard AlphaScreen® *SureFire®* protocol, or (B) for phospho-Chk2 by Western blot, or (C) for Chk2 by Western blot.



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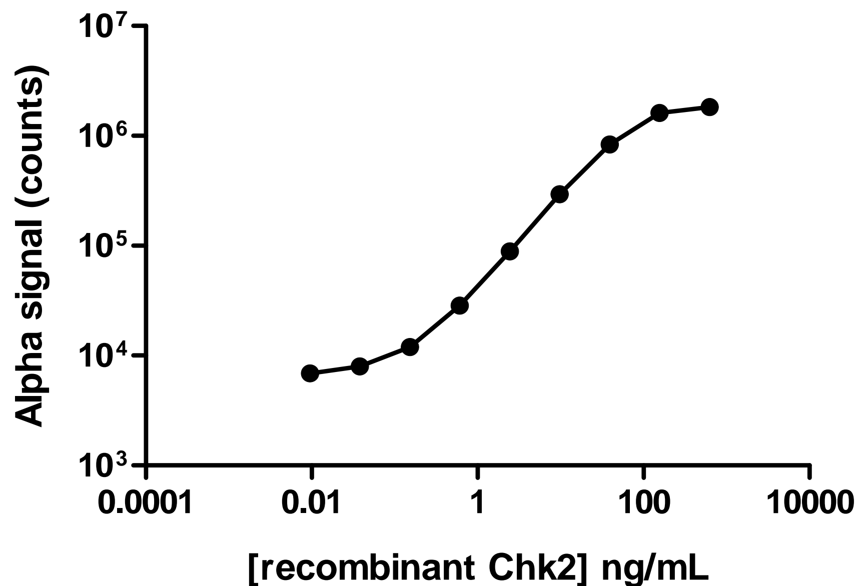


Recombinant active human Chk2 (R&D Systems Cat# 1358-KS-010) was diluted to various concentrations in 1x Lysis buffer containing 0.1% IgG-free BSA (Jackson Cat# 001-000-161), and analyzed using the standard 2-step AlphaScreen® *SureFire*® protocol for p-Chk2 detection. Under these conditions, the detection limit for this analyte was less than 1ng/mL.



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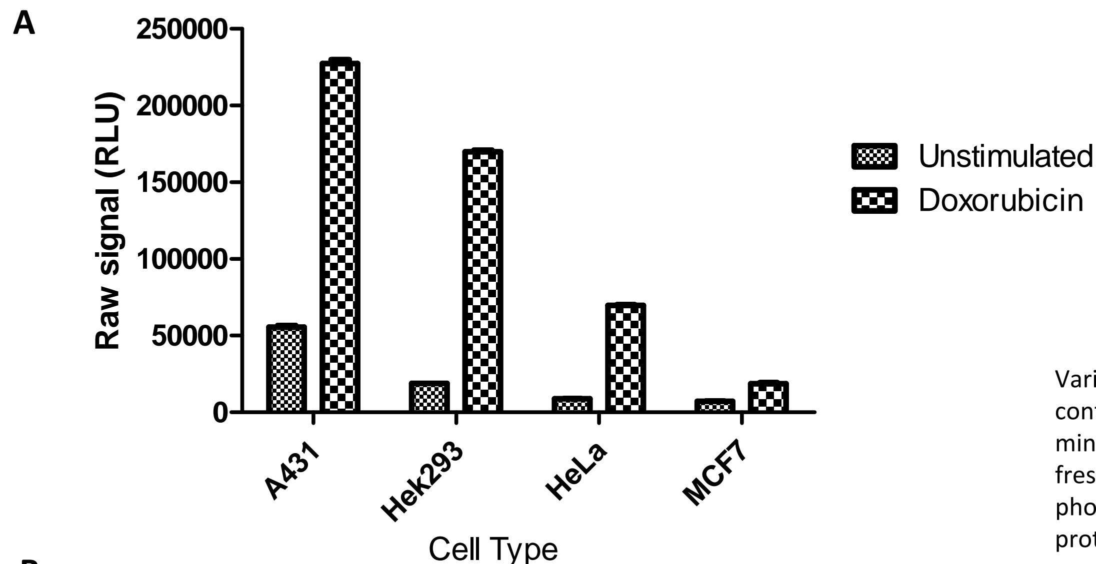


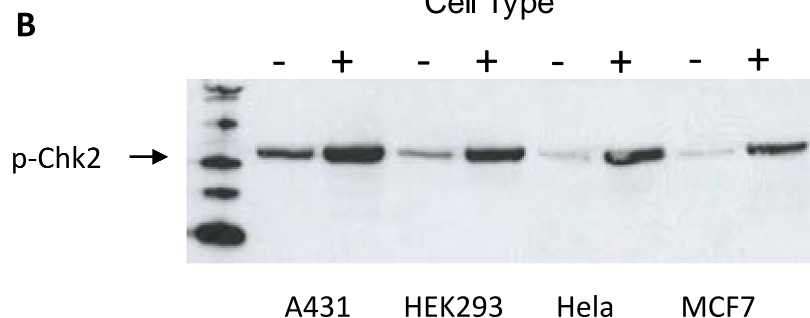


Various cells were grown in T25 flasks until approximately confluent, and either stimulated with doxorubicin for 60 minutes or left unstimulated. Cells were lysed with 2mL of freshly prepared 1X Lysis buffer, and were assayed for (A) phospho-Chk2 using the standard AlphaScreen® *SureFire*® protocol, or (B) for phospho-Chk2 by Western blot.



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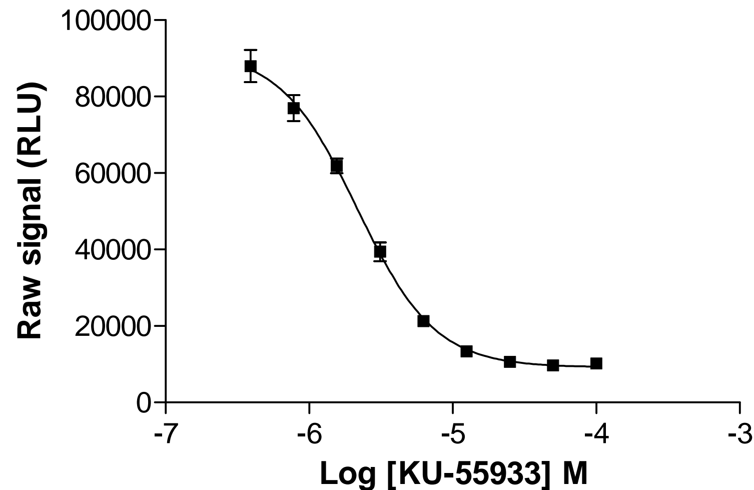
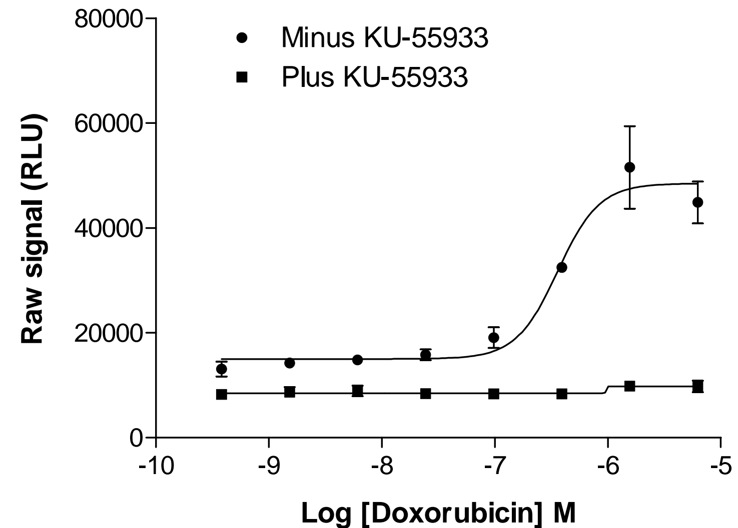



HEK293 cells were seeded into 96 well plates at a density of 20K/well in media + 10% FBS, and incubated overnight. The media was removed from the wells, and replaced with serum-free media containing various concentrations of the ATM-kinase inhibitor KU-55933 for 60 minutes. The media was removed, and the cells were lysed with 30µL of freshly prepared 1X Lysis buffer with shaking for 10 minutes. A portion of lysate (4µL) was transferred to a proxiplate and analysed for p-Chk2 using the standard AlpahScreen® *SureFire*® protocol.




A431 cells were seeded into 384 well proxiplates at a density of 5K/well in 10µL media + 10% FBS, and incubated overnight. The cells were then either treated with 5µL serum-free media containing ATM-kinase inhibitor KU-55933 for 60 minutes, or media alone. Finally, the cells were stimulated with 5µL of various concentrations of doxorubicin diluted in serum-free media for 90 minutes. The media was removed, and the cells were lysed with 4µL of freshly prepared 1X Lysis buffer with shaking for 10 minutes. The lysate was analysed for p-Chk2 using the standard AlpahScreen® *SureFire*® protocol.



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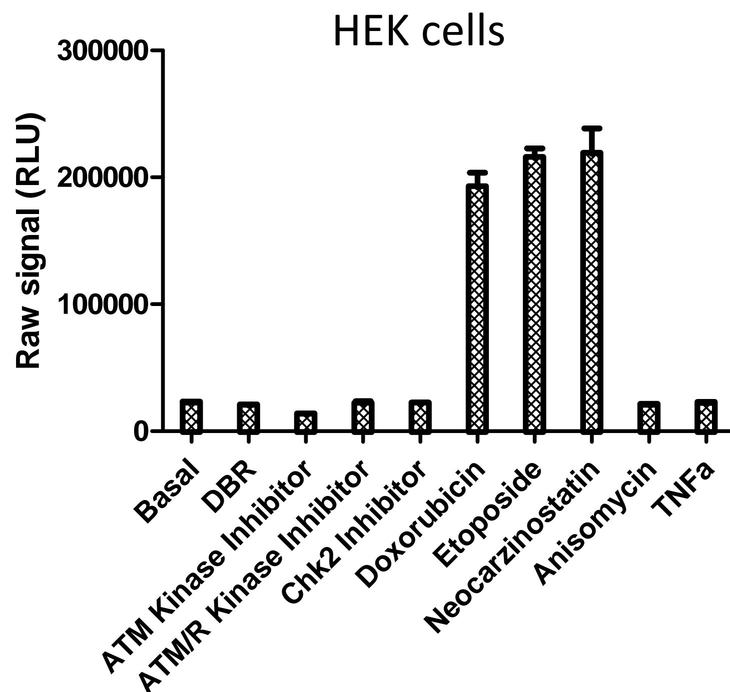
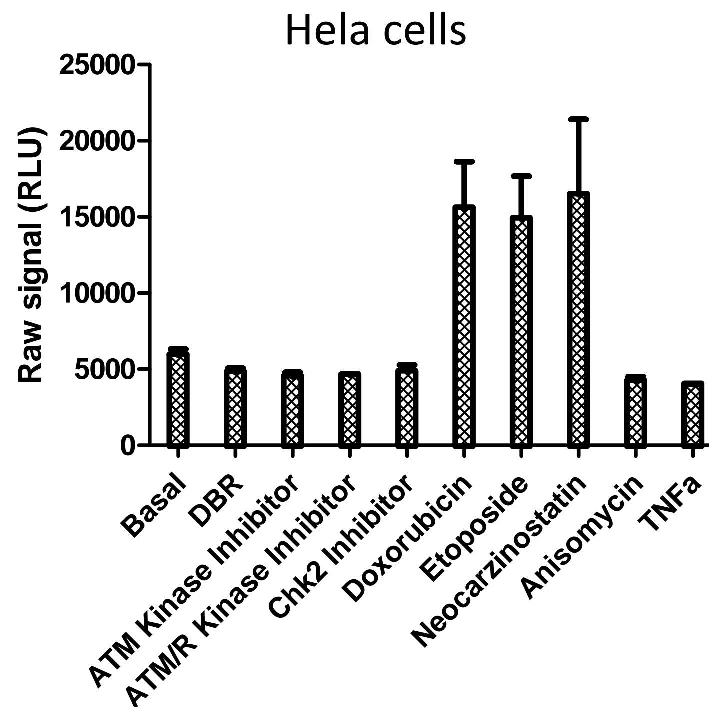



HEK293 cells were plated in 12 well plates at a density of 400K/well in media containing 10% FBS, and incubated overnight. The following day, the media was replaced with fresh serum-free media containing various treatments, and incubated for 2 hours. The media was removed, and the wells were lysed with 250µL freshly prepared 1X Lysis buffer with shaking for 10 minutes. A portion of lysate 4µL was transferred to a proxiplate and analysed for p-Chk2 using the standard AlphaScreen® *SureFire*® protocol.




Hela cells were plated in 12 well plates at a density of 200K/well in media containing 10% FBS, and incubated overnight. The following day, the media was replaced with fresh serum-free media containing various treatments, and incubated for 2 hours. The media was removed, and the wells were lysed with 250µL freshly prepared 1X Lysis buffer with shaking for 10 minutes. A portion of lysate 4µL was transferred to a proxiplate and analysed for p-Chk2 using the standard AlphaScreen® *SureFire*® protocol.



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