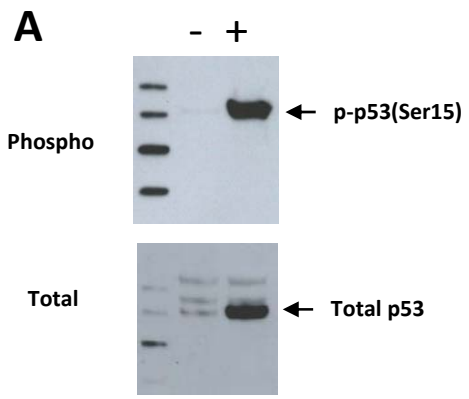
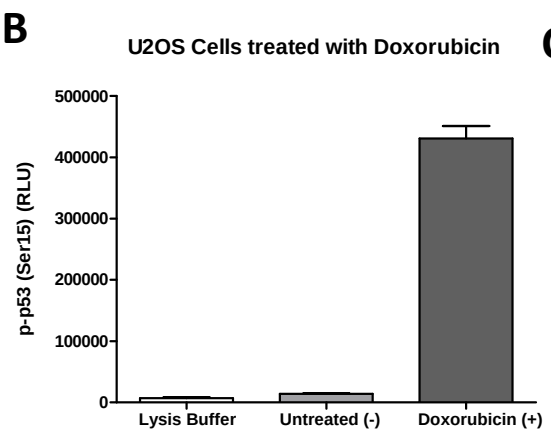
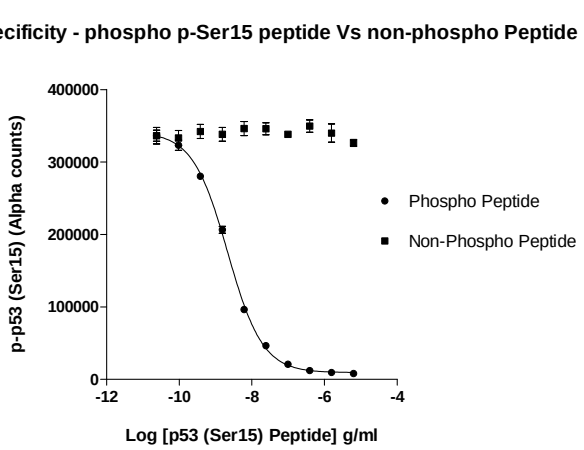
AlphaScreen® SureFire® phospho-p53 (Ser15) Assay Kit

Catalog #
TGRP53S500
TGRP53S10K
TGRP53S50K

p53 (also known as protein 53 or tumor suppressor protein 53), is a transcription factor that plays a major role in regulating cellular response to DNA damage and other genomic aberrations. p53 becomes activated in response to several types of cellular stress, including DNA damage, oxidative stress and osmotic shock. Phosphorylation of the N-terminus of p53, a region that contains a large number of phosphorylation sites, impairs the ability of its negative regulator, MDM2, to interact with p53. When activated, the half-life of the p53 protein is increased, leading to its accumulation in cells. Activated p53 also undergoes conformational change, allowing tetramerization. Activation of p53 can ultimately lead to either cell cycle arrest and DNA repair, or apoptosis.







Cell Handling Protocol:
2 x T175 Flasks of U2OS cells were grown until 80% confluent, then treated with (+) 1µM Doxorubicin for 18 hours at 37°C, or left untreated. The media was then removed from the flasks and the cells were lysed in 8mL freshly prepared 1x Lysis Buffer with shaking for 10 minutes.

Assay Protocol:
The lysates were analyzed for **(A)** phospho-p53 (Ser15) and Total p53 or by Western Blot (20µL lysate per lane) using the same antibodies used for the AlphaScreen® *SureFire*® Reaction buffer. **(B)** The same lysates were also analyzed using the standard 2-plate AlphaScreen® *SureFire*® protocol at room temperature (4µL lysate per 384-Proxiplate well). **(C)** To confirm the specificity of the assay for phospho-p53, a peptide surrounding Ser15 was generated, and either phosphorylated or not. This peptide was serially diluted into the U2OS lysates, transferred to a 384-well Proxiplate™ and analyzed for p-p53 (Ser15) using the standard 2-plate AlphaScreen® *SureFire*® protocol. Only the peptide phosphorylated at Ser15 is able to inhibit the assay signal, while the corresponding peptide without phosphorylation does not.

AlphaScreen® SureFire® phospho-p53 (Ser15) Assay Kit

Catalog #
TGRP53S500
TGRP53S10K
TGRP53S50K

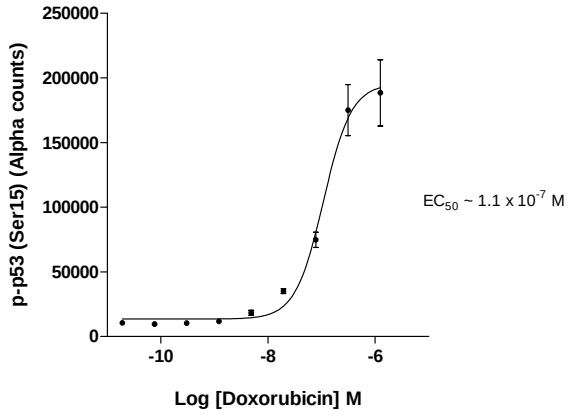
Cell Handling Protocol:
U2OS Cells (Passage 37) were seeded at 25K/cells/well in 96-well microplates, in media containing 10% FBS. Cells were incubated overnight at 37°C.

Assay Protocol:
The following day the media was removed and the cells were dose stimulated with various concentrations of either Doxorubicin **(A)** or Etopiside **(B)** for 18 hours at 37°C. The media was removed from the wells, and the cells were lysed with 50 µL/well 1X Lysis buffer with shaking for 10 minutes.

Lysates (4µL) were transferred to a 384-well Proxiplate™ and analyzed for phospho-p53 (Ser15) using the standard 2-plate AlphaScreen® *SureFire*® protocol.

A

Doxorubicin-mediated phospho-p53 (Ser15) in U2OS cells



B

Etoposide-mediated phospho-p53 (Ser15) in U2OS Cells