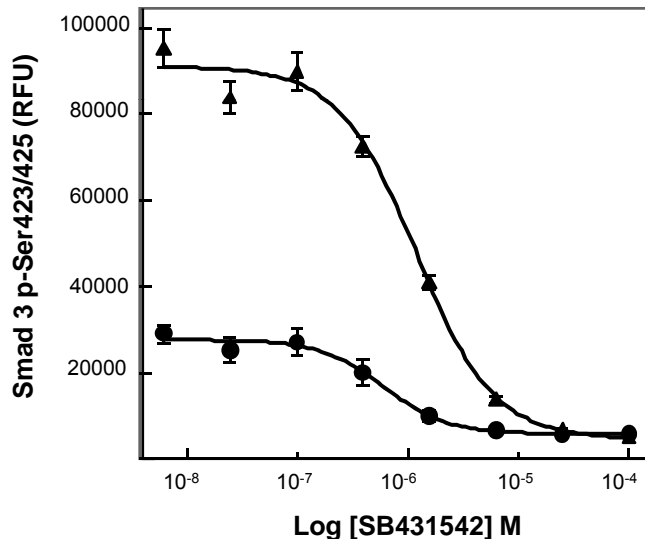


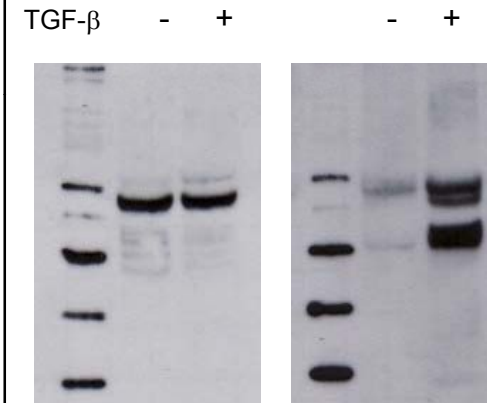
TGRSM3S500TK
TGRSM3S500
TGRSM3S10K
TGRSM3S50K

AlphaScreen® SureFire® phospho-Smad-3 (Ser423/425) Kit

The Smad family of signal transduction molecules are components of the TGF- β signaling pathway. There are three classes of Smads: receptor-regulated Smads, which include Smad1, 2, 3, 5 and 8 (R-Smads), the common-mediator Smad4 (Co-Smad), and the antagonistic or inhibitory Smads, Smad6 and 7 (I-Smads). Following stimulation by TGF- β , Smad3 becomes phosphorylated at the carboxyl termini (Ser423 and 425) by TGF- β RI. Phosphorylated Smad 3 complexes with Smad4, translocates to the nucleus and regulates gene expression.



Hela cells were seeded at 30,000 cells/well into 96-well microplates in media containing 10% FBS. The following day the media was removed and the cells were starved in serum-free media overnight. The following day the cells were incubated with various concentrations of the TGF- β Receptor inhibitor SB431542 for 40 min. The cells were then either stimulated with 10 ng/mL TGF- β for 60 min, or left unstimulated. The cells were lysed with 50 μ L of freshly prepared 1X Lysis buffer for 10 min with gentle shaking, and analyzed for phospho-Smad3 using the standard AlphaScreen SureFire protocol.



Western blot of Hela cells lysates from flasks of cells either stimulated with 10 ng/mL TGF- β for 60 min (+), or left unstimulated (-), using antibodies against total-Smad3 (left panel), or phospho-Smad3 (right panel). Western blots were developed using Western Lightning™ Plus-ECL (Perkin Elmer Cat.# NEL05001EA).