

AlphaLISA® SureFire® Ultra™

Human and Mouse p-SMAD3 (Ser423/425) High Specificity Detection Kit

Product number: ALSU-PSM3-B500, ALSU-PSM3-B10K,
 ALSU-PSM3-B50K, ALSU-PSM3-B-HV



Kit specificity:

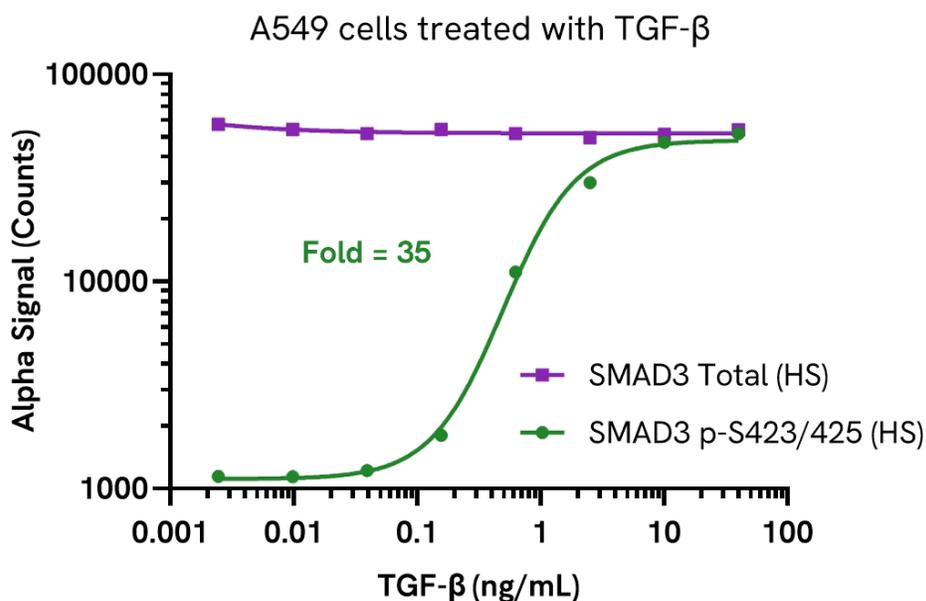
This assay kit contains antibodies which recognize the phospho-Ser423/425 epitope and a distal epitope on SMAD3. The protein detected by this kit corresponds to UniProt ID P84022. SMAD3 is also known as Mothers against decapentaplegic homolog 3, JV15-2 and MAD homolog 3. These antibodies recognize SMAD3 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

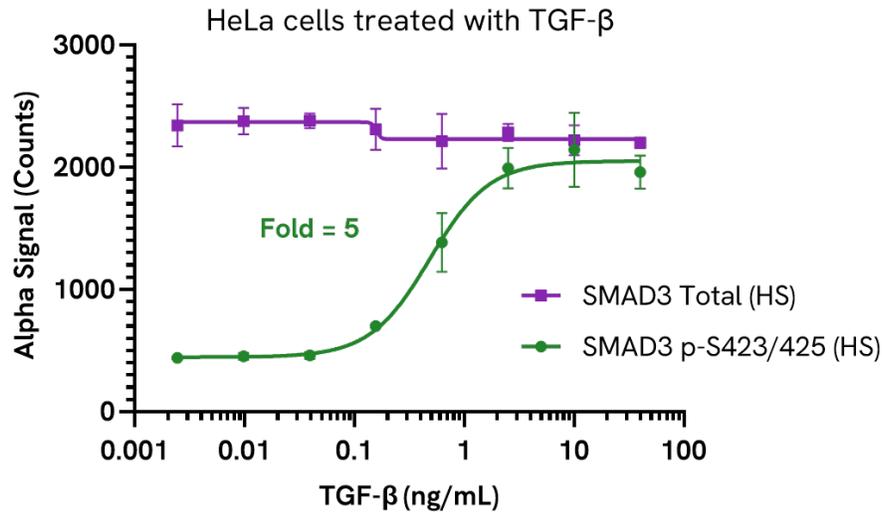
Positive Control Lysate: Prepared from RAW 264.7 cells cultured to confluence in T175 flasks in 10% FBS containing medium, treated with 50 ng/mL TGF-β for 90 minutes and lysed with 6 mL of Lysis Buffer.

Representative data:

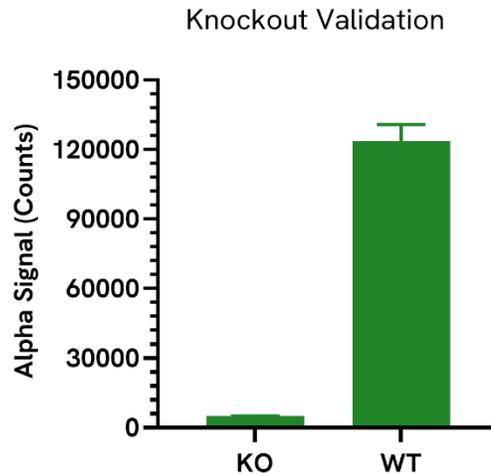
Data obtained with a 2-plate, 2-incubation protocol. A549 cells were seeded at 40K cells/well in a 96-well plate and incubated overnight. Cells were starved for 1 hour and then treated with increasing concentrations of TGF-β for 90 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser423/425) and Total SMAD3 using the SureFire Ultra High Specificity kits. Equivalent to approximately 2,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. HeLa cells were seeded at 40K cells/well in a 96-well plate and incubated overnight. Cells were treated with increasing concentrations of TGF- β for 90 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser423/425) and Total SMAD3 using the *SureFire Ultra High Specificity* kits. Equivalent to approximately 2,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. A549 wild type (WT) and SMAD3 knockout (KO, Abcam ab263915) cells were grown to confluency in T175 flasks, starved for 1 hour, and stimulated with 50 ng/mL TGF- β for 90 minutes. Cells were lysed with Lysis Buffer and assayed for Phospho (Ser423/425) SMAD3 using the *SureFire Ultra High Specificity* kit.



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