AlphaLISA® *SureFire® Ultra™*

Human and Mouse SMAD3 Total Detection Kit

Product number: ALSU-TSM3-A500, ALSU-TSM3-A10K, ALSU-TSM3-A50K, ALSU-TSM3-A-HV

Kit specificity:

This assay kit contains antibodies which recognize distinct epitopes 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.

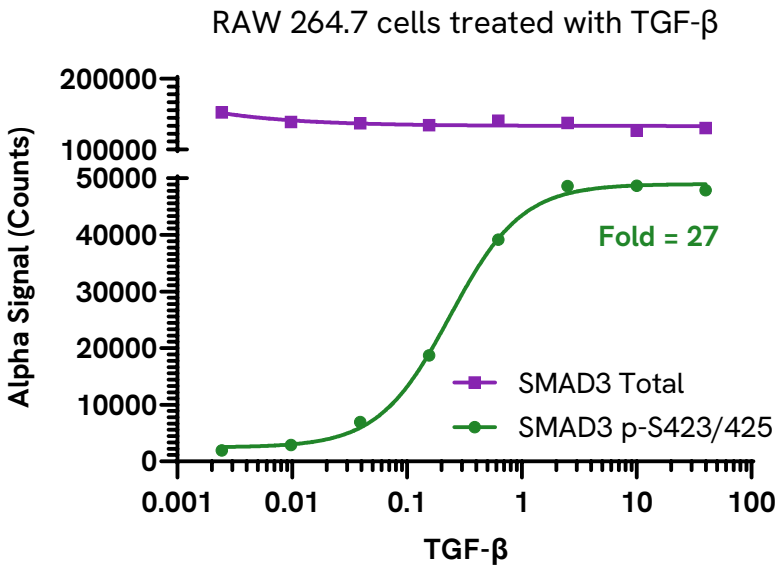
Important specificity note: Based on the antibody characteristics used in this assay and the knockout validation data presented, the assay preferentially recognizes SMAD3 but may exhibit some cross-reactivity with SMAD2. If SMAD3-specific detection is required, we recommend the High Specificity Total SMAD3 assay (ALSU-TSM3-B).

Control lysate information:

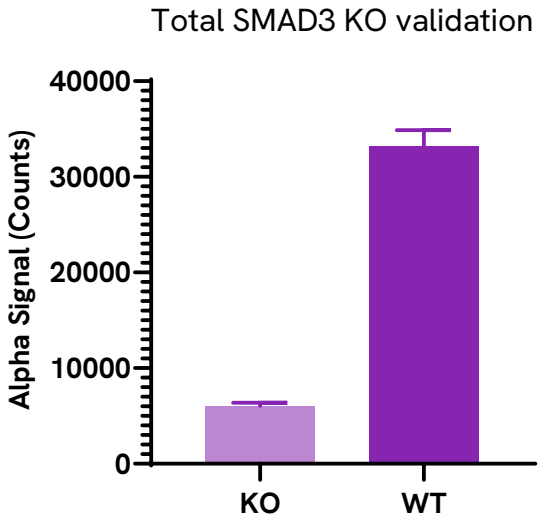
Positive Control Lysate: Prepared from RAW 264.7 cells, cultured to confluence in T175 flasks in 10% FBS containing media, then treated with 50 ng/mL TGF-β for 90 minutes and lysed with 6 mL of Lysis Buffer.

Representative data

Data obtained with a 2-plate, 2-incubation protocol. RAW 264.7 cells were plated at 20K cells/well in a 96 well plate and incubated for two days. Cells were serum-starved for 60 minutes then treated with TGF-β at the indicated concentrations for 90 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser423/425) and Total SMAD3 using respective *SureFire Ultra* kits. Equivalent to approximately 2,000 cells/datapoint.



Knockout validation of SMAD3 Total Assay.
A549 wild type (WT) and SMAD3 knockout (KO, Abcam ab263915) cells were grown to confluency in T175 flasks. Cells were lysed with Lysis Buffer and assayed for Total SMAD3 by *SureFire Ultra*. Equivalent to approximately 6,000 cells/datapoint. This data demonstrates that the Total SMAD3 assay preferentially recognizes SMAD3 but may exhibit some cross-reactivity with SMAD2 since some signal was detected in the KO cell line.



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