AlphaLISA® *SureFire® Ultra™*

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

Product number: ALSU-PSM3-A500, ALSU-PSM3-A10K, ALSU-PSM3-A50K, ALSU-PSM3-A-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.

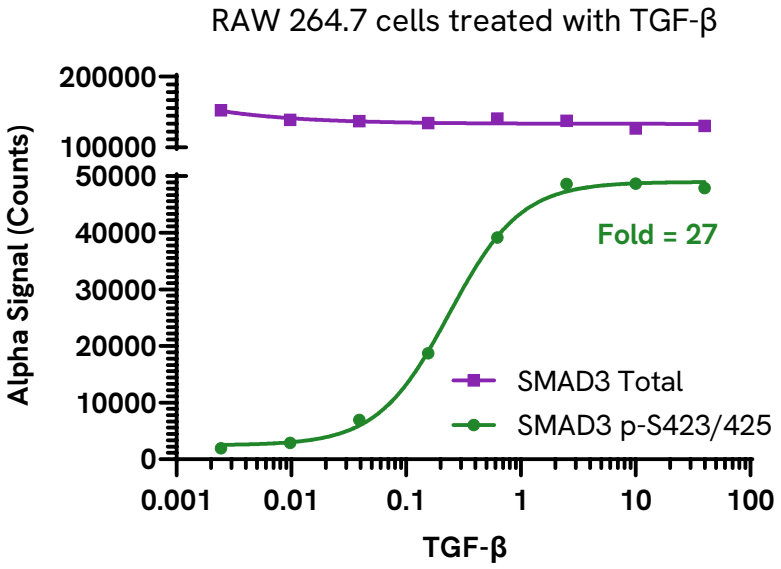
Important specificity note: Based on the antibody characteristics used in this assay and the knockout validation data presented, the assay preferentially recognizes phospho-SMAD3 but may exhibit some cross-reactivity with phospho-SMAD2. If SMAD3-specific detection is required, we recommend the High Specificity Phospho-SMAD3 assay (ALSU-PSM3-B).

Control lysate information:

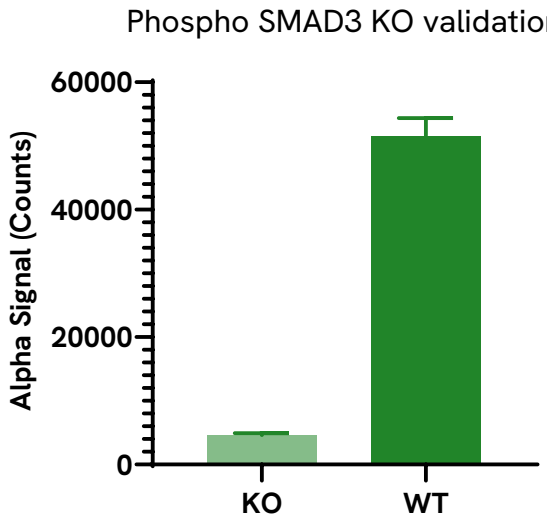
Positive Control Lysate: Prepared from RAW 264.7 cells, cultured to confluence in T175 flasks in 10% FBS containing medium, then treated with 50 ng/mL TGF-β for 90 minutes and lysed with 6 mL of Lysis Buffer.

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 48 hours. Cells were serum starved for 1 hour 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 Phospho Assay.
A549 wild type (WT) and SMAD3 knockout (KO, Abcam ab263915) cells were grown to confluency in T175 flasks. Cells were starved for 1 hour and then treated with 50 ng/mL of TGF-β for 90 minutes. Cells were lysed with Lysis Buffer and assayed for Phospho (Ser423/425) SMAD3 by SureFire Ultra. Equivalent to approximately 6,000 cells/datapoint. This data demonstrates that the Phospho SMAD3 assay preferentially recognizes phospho-SMAD3 but may exhibit some cross-reactivity with phospho-SMAD2 since some signal was detected in the KO cell line.



The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF"

Copyright ©2026, Revvity, Inc. All rights reserved.