Human Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) Antibody

Polyclonal Rabbit Serum

Catalog Number: AB3226

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Phospho-Smad2/3 when dually phosphorylated at S465/S467 and S423/S425, respectively.
Source	Polyclonal Rabbit Serum
Purification	N/A
Immunogen	Phosphopeptide containing human Smad3 S423/S425 sites
Formulation	Lyophilized from rabbit serum in PBS and Sodium Azide. See Certificate of Analysis for details.

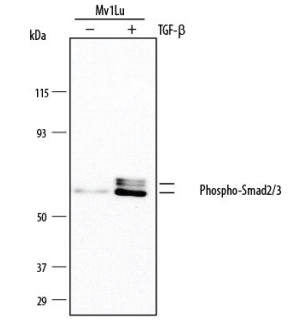
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1:500 dilution	See Below
Immunocytochemistry	1:50-1:100 dilution	See Below
Simple Western	1:50-1:100 dilution	See Below

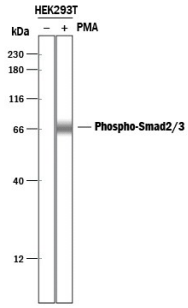
DATA

Western Blot



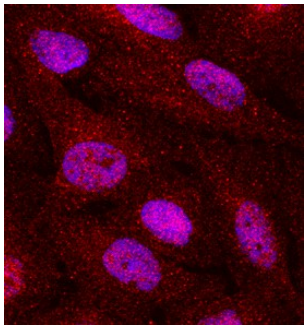
Detection of Human Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) by Western Blot. Western blot shows lysates of Mv1Lu mink lung epithelial cell line untreated (-) or treated (+) with 10 ng/mL Recombinant Human TGF-β2 (Catalog # 302-B2) for 24 hours. PVDF membrane was probed with 1:500 dilution of Rabbit Anti-Human Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) Polyclonal Antibody (Catalog # AB3226), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific bands were detected for Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) at approximately 60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western



Detection of Human Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) by Simple Western™. Simple Western lane view shows lysates of HEK293T human embryonic kidney cell line untreated (-) or treated (+) with 200 nM PMA for 20 minutes, loaded at 0.5 mg/mL. A specific band was detected for Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) at approximately 68 kDa (as indicated) using 1:50-1:100 dilution of Rabbit Anti-Human Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) Polyclonal Antibody (Catalog # AB3226). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Immunocytochemistry



Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) in Mv1Lu Mink Cell Line. Smad2/3 phosphorylated at S465/S467 and S423/S425 was detected in immersion fixed Mv1Lu mink lung epithelial cell line treated with 10 ng/mL of Recombinant Human TGF-beta 2 (Catalog # 302-B2) using Rabbit Anti-Human Phospho-Smad2 (S465/S467)/ Smad3 (S423/S425) Polyclonal Antibody (Catalog # AB3226) at 1:50-1:100 dilution for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute with 250 µL sterile PBS containing 0.02% Sodium Azide.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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Polyclonal Rabbit Serum
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BACKGROUND

Smad3 (mothers against DPP homolog 3) is a transcription factor that functions downstream of the transforming growth factor β receptor (TGF-β R). Smad3 is phosphorylated in cells stimulated with TGF-β, complexes with Smad4, and translocates to the nucleus to upregulate gene transcription. Smad3 is critical for signaling fibrosis and wound healing.

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