Phospho-SMAD2 (Ser465, Ser467) Recombinant Rabbit Monoclonal Antibody (8H1L19)

Product Details	
Size	100 µg
Species Reactivity	Human
Host/Isotype	Rabbit / IgG
Class	Recombinant Monoclonal
Type	Antibody
Clone	8H1L19
Conjugate	Unconjugated
Immunogen	Synthetic peptide corresponding to human SMAD2, aa 461-467, phosphorylated at Serine 465/467
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS
Contains	0.09% sodium azide
Storage conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.
RRID	AB_2532493

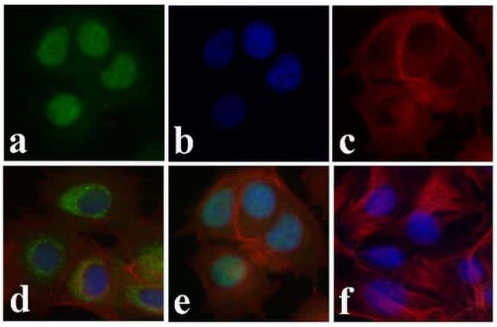
Applications	Tested Dilution	Publications
Western Blot (WB)	1-2 µg/mL	-
Immunocytochemistry (ICC/IF)	3-5 µg/mL	-
Immunoprecipitation (IP)	Assay-dependent	-
RNA Immunoprecipitation (RIP)	Assay-dependent	-

Product Specific Information

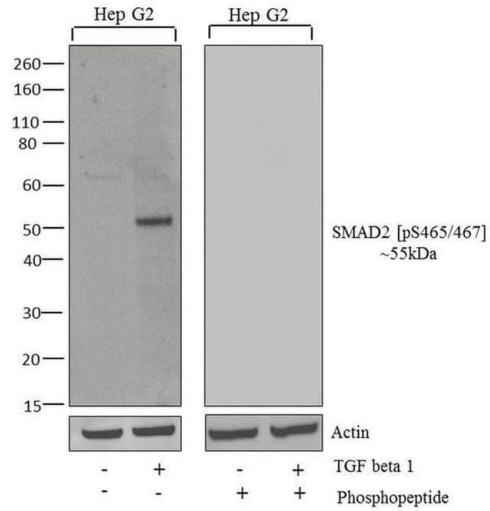
This antibody is predicted to react with mouse, bovine, zebrafish and drosophila based on sequence homology.

Recombinant rabbit monoclonal antibodies are produced using in vitro expression systems. The expression systems are developed by cloning in the specific antibody DNA sequences from immunoreactive rabbits. Then, individual clones are screened to select the best candidates for production. The advantages of using recombinant rabbit monoclonal antibodies include: better specificity and sensitivity, lot-to-lot consistency, animal origin-free formulations, and broader immunoreactivity to diverse targets due to larger rabbit immune repertoire.

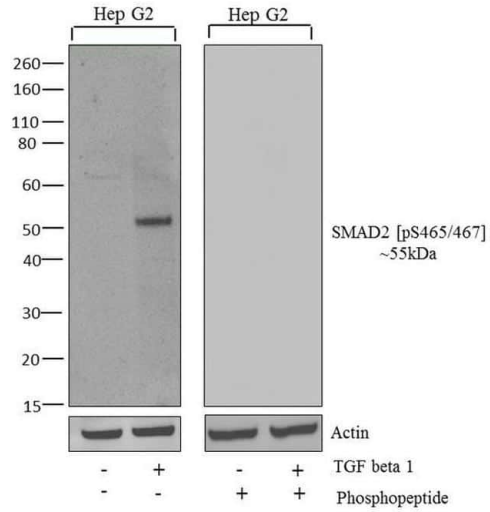
Product Images For Phospho-SMAD2 (Ser465, Ser467) Recombinant Rabbit Monoclonal Antibody (8H1L19)



Phospho-SMAD2 (Ser465, Ser467) Antibody (701582) in ICC/IF
Immunofluorescent analysis of SMAD2 (pSpS465/467) was done on 70% confluent log phase HepG2 cells. The cells were fixed with 4% paraformaldehyde for 15 minutes; permeabilized with 0.25% Triton X-100 for 10 minutes followed by blocking with 5% BSA for 1 hour at room temperature. The cells were incubated with SMAD2 (pSpS465/467) Recombinant Rabbit Monoclonal Antibody (Product # 701582) at a dilution of 1:400 in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor® 488 Goat anti-Rabbit IgG Secondary Antibody (Product # A-11008) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 594 Phalloidin (Product # A12381). Panel d is an untreated HepG2 cells showing nuclear localization. Upon treatment SMAD2 (pSpS465/467) shows nuclear translocation (Panel e). Panel f shows no primary antibody control. The images were captured at 20X magnification.



Phospho-SMAD2 (Ser465, Ser467) Antibody (701582)
Altered expression of proteins upon cell treatment demonstrates antibody specificity. Western blot of SMAD2 pS465pS467 using Anti-SMAD2 (pS465pS467) Recombinant Rabbit Monoclonal Antibody (Product # 701582), shows increased expression of SMAD2 [pS465pS467] in HepG2 cell line upon TGF beta treatment. {TM}



Phospho-SMAD2 (Ser465, Ser467) Antibody (701582) in WB
Western blot analysis was performed to detect SMAD2 (pSpS465/467) protein by loading 30 µg of Hep G2 and Hep G2 treated with TGF beta 1 (20nM for 30 minutes; lane1, 2) cell lysates using a Novex®NuPAGE®4-12% Bis-Tris gel (Product # NP0321BOX), XCell SureLock Electrophoresis System (Product # EI0002), Novex® Sharp Pre-Stained Protein Standard (Product # LC5800) and iBlot® Dry Blotting System (Product # IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5% skim milk for 1 hour at room temperature. To confirm specificity; competition was performed with phosphopeptide (10 µg/mL, preincubated with antibody 30 minutes (lane 3 and 4).SMAD2 (pSpS465/467) was detected at ~55 kDa using SMAD2 (pSpS465/467) Recombinant Rabbit Monoclonal Antibody (Product # 701582) at 1:500 dilution in 2.5% skim milk at 4°C; incubated overnight on a rocking platform. Goat anti-Rabbit IgG-RP Secondary Antibody (Product # G-21234) was used at 1:5000 dilution and chemiluminescent detection was performed using Pierce™ ECL Western blotting Substrate (Product # 32106).

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