abcam

Product datasheet

Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] ab92698

Recombinant RabMAb

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Overview

Product name	Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3]
Description	Rabbit monoclonal [MMC-1-104-3] to Smad1 (phospho S463 + S465) + SMAD5 (phospho S463 + S465) + SMAD9 (phospho S465 + S467)
Host species	Rabbit
Specificity	This antibody may cross-react with Smad1 Phospho (pS463/465) and Smad9 Phospho (pS465/467). Stimulation may be required to allow detection of the phosphorylated protein. Please see images below for recommended treatment conditions and positive controls.
Tested applications	**Suitable for:** Dot blot, WB, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa cell lysate treated with BMP-4. IHC-P: Human breast carcinoma, colonic carcinoma, tonsil, hepatocellular carcinoma, cervical carcinoma and glioma tissues.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	MMC-1-104-3
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92698 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

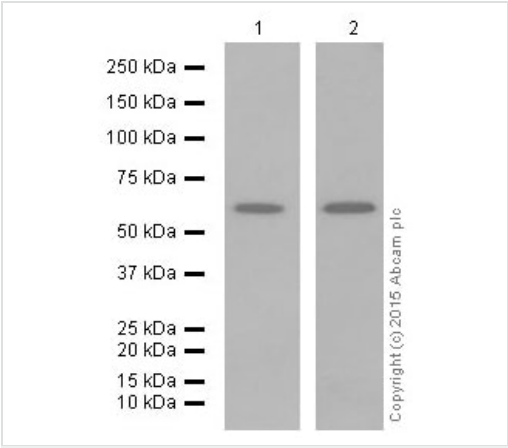
Application	Abreviews	Notes
Dot blot		1/1000.
WB	★★★★☆ **(1)**	1/1000 - 1/10000. Predicted molecular weight: 52 kDa.
IHC-P		1/100 - 1/800. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Cellular localization Smad1: Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4. Co-localizes with LEMD3 at the nucleus inner membrane. SMAD5: Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4. SMAD9: Cytoplasm. Nucleus. In the cytoplasm in the absence of ligand. Migration to the nucleus when complexed with SMAD4.

Images



Western blot - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

All lanes : Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698) at 1/5000 dilution (purified)

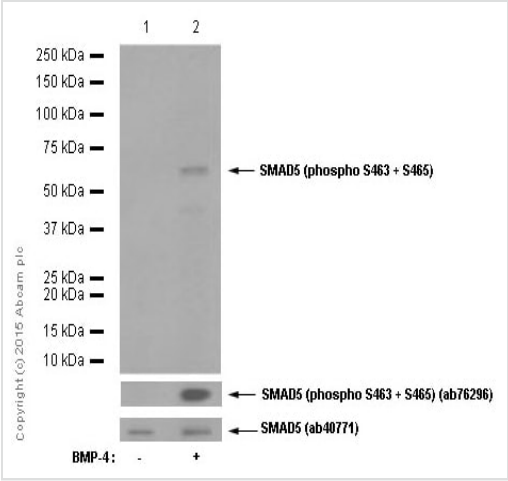
Lane 1 : Mouse brain tissue lysate
Lane 2 : Rat brain tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 52 kDa
Observed band size: 58 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



Western blot - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

All lanes : Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698) at 1/10000 dilution (purified)

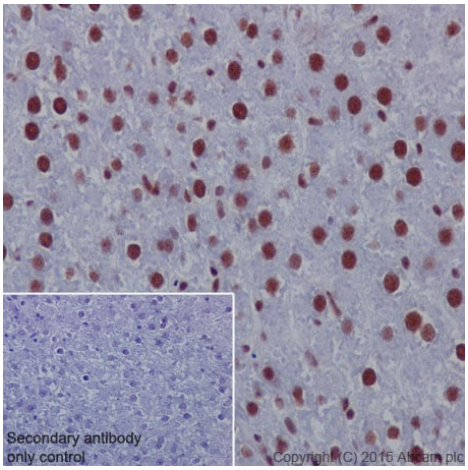
Lane 1 : HeLa whole cell lysate - untreated
Lane 2 : HeLa whole cell lysate - treated with Bone Morphogenetic protein-4

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

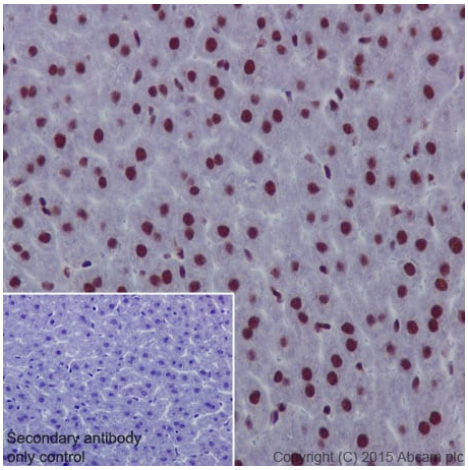
Predicted band size: 52 kDa
Observed band size: 58 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



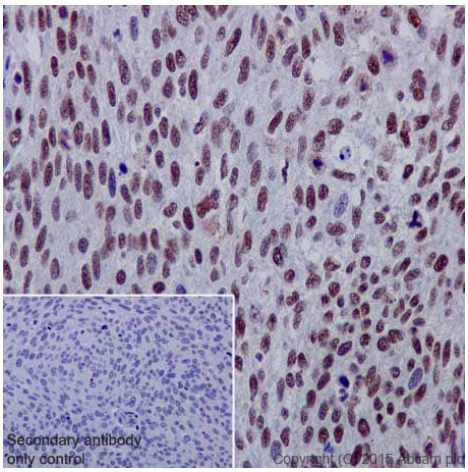
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue labelling SMAD5 (phospho S463 + S465) with purified ab92698 at a dilution of 1/800. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



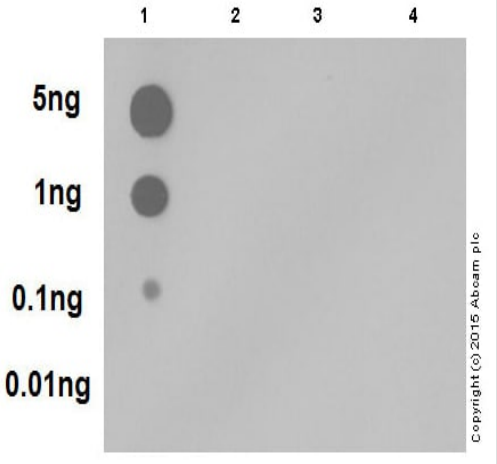
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue labelling SMAD5 (phospho S463 + S465) with purified ab92698 at a dilution of 1/800. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling SMAD5 (phospho S463 + S465) with purified ab92698 at a dilution of 1/800. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

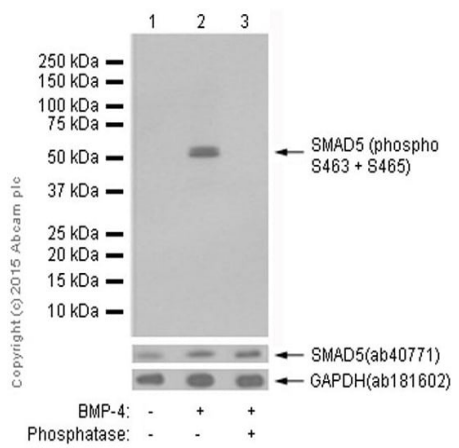


Dot Blot - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Dot blot analysis of SMAD5 (pS463 + pS465) peptide (Lane 1), SMAD5 (pS465) peptide (Lane 2), SMAD5 (pS463) peptide (Lane 3) and SMAD5 non-phospho peptide (Lane 4) labelling SMAD5 (pS465) with purified ab92698 at a dilution of 1/1000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

All lanes : Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698) at 1/10000 dilution

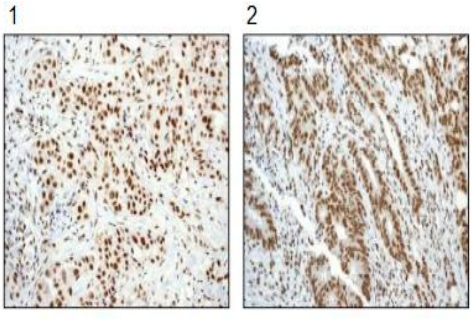
Lane 1 : Untreated HeLa (human cervix adenocarcinoma) whole cell lysates 20µg
Lane 2 : HeLa (human cervix adenocarcinoma) treated with Bone Morphogenetic Protein-4 (BMP-4) whole cell lysates 20µg
Lane 3 : HeLa (human cervix adenocarcinoma) treated with Bone Morphogenetic Protein-4 (BMP-4) and phosphatase, whole cell lysates 20µg.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 52 kDa
Observed band size: 58 kDa

Exposure time: 30 seconds

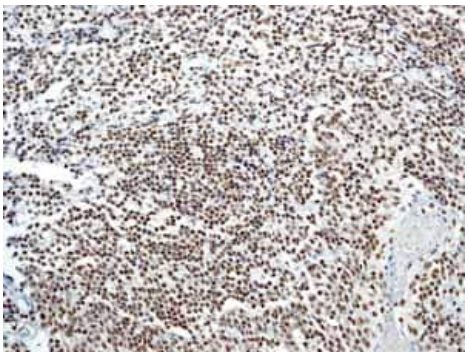
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of (1) human breast carcinoma and (2) human colonic carcinoma tissues labelling SMAD5 (phospho S463 + P465) with unpurified ab92698 at a dilution of 1/100.

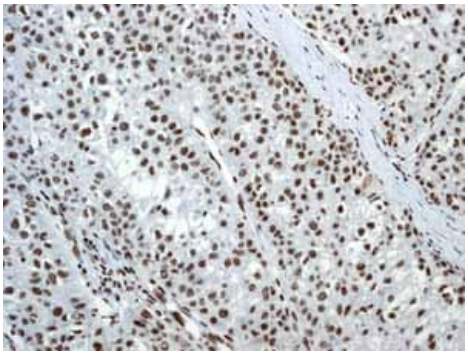
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal human tonsil tissue labelling SMAD5 (phospho S463 + S465) with unpurified ab92698.

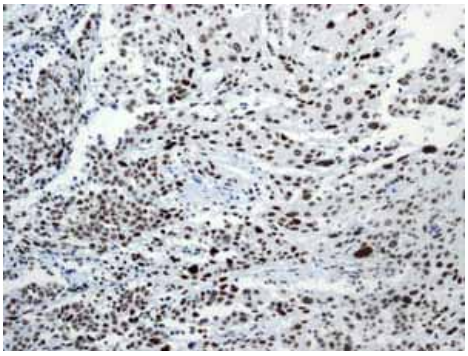
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human hepatocellular carcinoma tissue labelling SMAD5 (phospho S463 + S465) with unpurified ab92698.

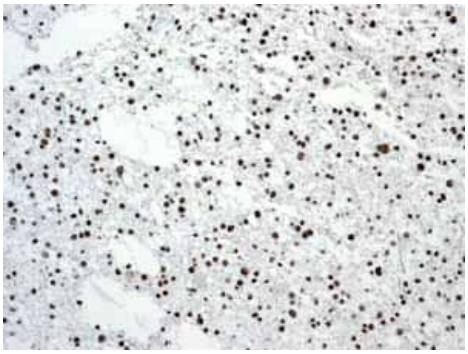
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling SMAD5 (phospho S463 + S465) with unpurified ab92698.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue labelling SMAD5 (phospho S463 + S465) with unpurified ab92698.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467) antibody [MMC-1-104-3] (ab92698)

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