

PRODUCT INFORMATION

Product name : SMAD5 antibody

Product type : Primary antibodies

Description : Rabbit polyclonal to SMAD5

Immunogen : 3 synthetic peptides (human) conjugated to KLH

Reacts with : Human, Mouse

Tested applications : ELISA, WB and IF

GENE INFORMATION

Gene Symbol : SMAD5

Gene Name : SMAD family member 5

Ensembl ID : ENSG00000113658

Entrez GeneID : 4090

GenBank Accession number : U59913

Swiss-Prot : Q99717

Molecular weight of SMAD5: 52.3kDa

Function : Transcriptional modulator activated by BMP (bone morphogenetic proteins) type 1 receptor kinase. SMAD5 is a receptor-regulated SMAD (R-SMAD).

Expected subcellular localization : Cytoplasm. Nucleus

APPLICATION NOTE

Recommended dilution :

- **ELISA:** Antibody specificity was verified by direct ELISA against the 3 immunogen peptides. A minimum titer of 1/ 3000 is determined. Appropriate specificity controls were run.
- **WB:** 1:1500
- **IF:** 1:1000

Optimal dilutions/concentration should be determined by the end user.

Raised in : Rabbit

Clonality : Polyclonal

Isotype : IgG

Purity : Purified Antibody

Storage buffer : 0.5 X PBS containing a final concentration of 50% Glycerol, 0.1% BSA and 0.01% Thimerosal.

Form : Liquid

Storage instruction : Store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

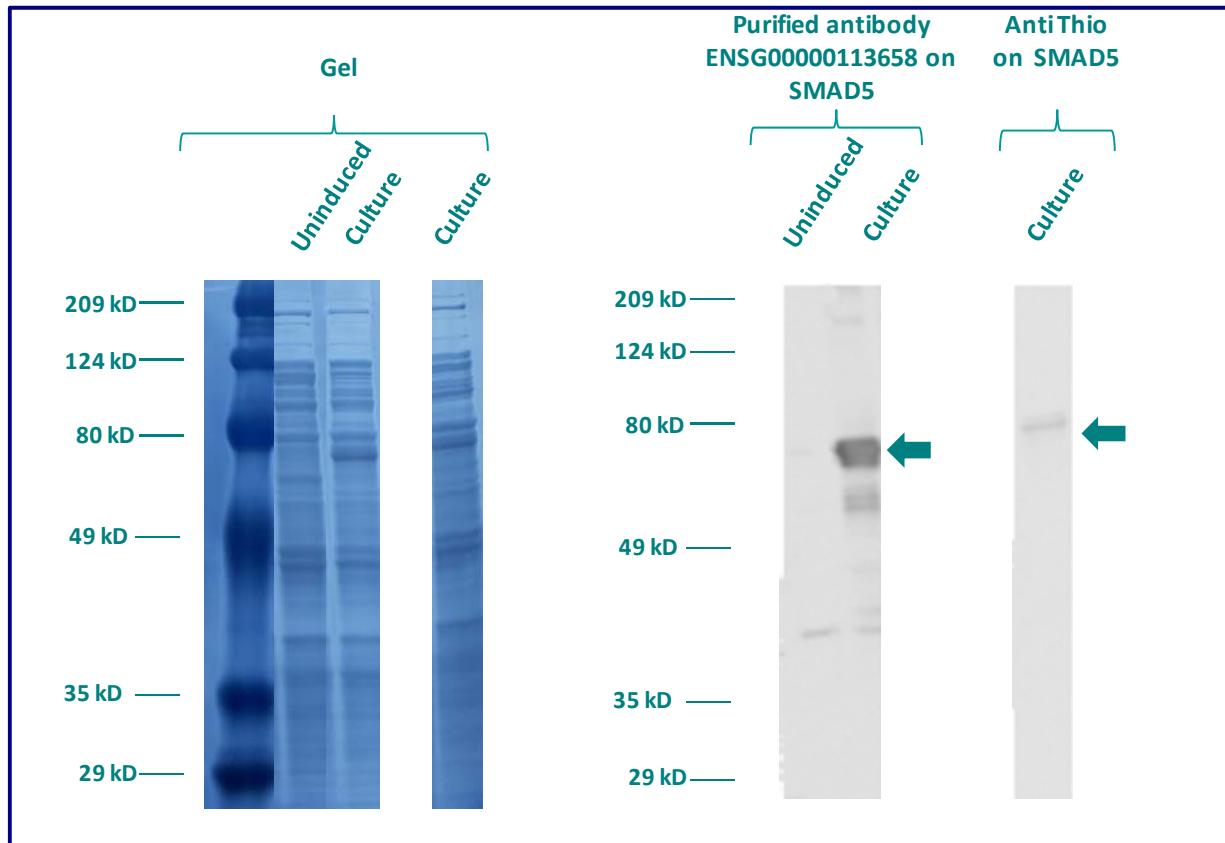
WESTERN BLOT ON RECOMBINANT PROTEIN

The purified antibody ENSG00000113658 has been tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

An anti-Thio (positive control) has been tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells) as a positive control.

Plasmid name : pBAD-DEST49.

Molecular weight of SMAD5 : 66.3kDa (52.3kDa + another 14kDa for the tag).



Gel concentration: 10%

Blocking: in 5% non-fat milk-PBST solution

1st Antibody: The antibodies are diluted in blocking buffer.

- Dilute the purified antibody ENSG00000113658 at 1: 1500
- Dilute the anti-thio at 1:5000

60 minutes of incubation

2nd Antibody: The antibody is diluted in blocking buffer.

- Dilute the anti-Rabbit IgG HRP conjugated at 1/10000
- 60 minutes of incubation

IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescence analysis of Mothers against decapentaplegic homolog 5 (SMAD5) expression in 5 cells lines (HELA, 293T/17, Capan-2, SH-SY5Y, Skin 3,44). The purified Antibody ENSG00000113658 has been tested at 1/1000.

Red staining : cytoskeleton (microtubules/ α -tubuline)

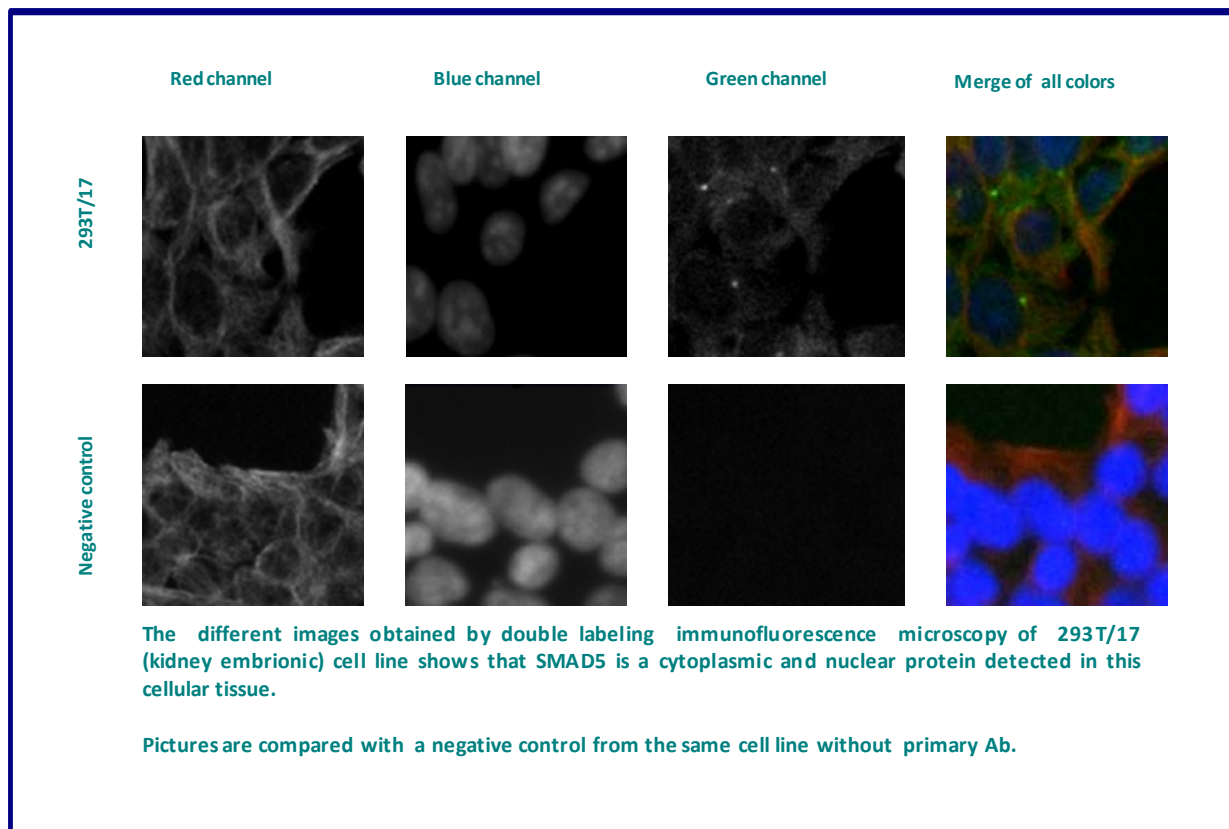
Blue staining : nucleus (Hoechst)

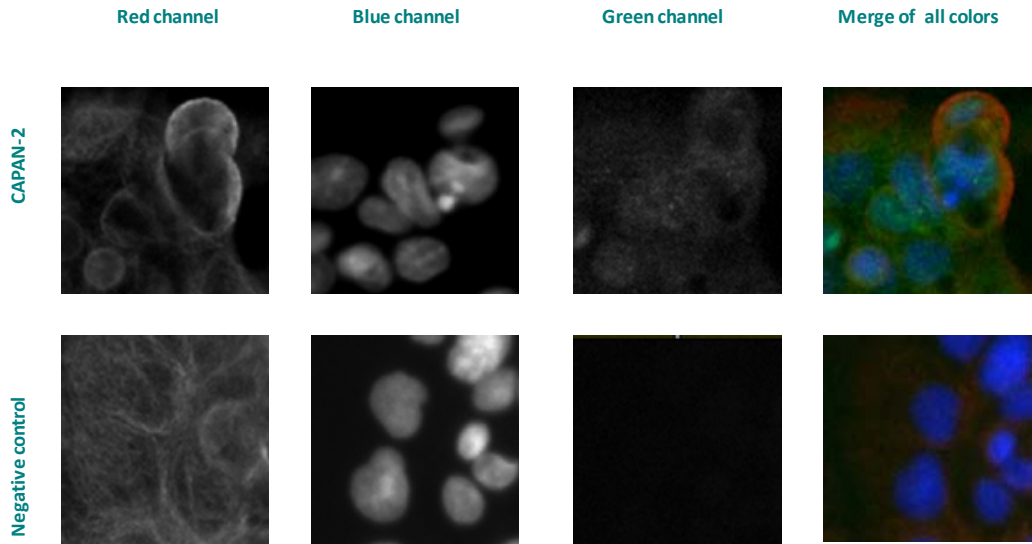
Green staining : anti- SMAD5 antibody (purified)

Expected subcellular location : Cytoplasm. Nucleus.

Note: Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4

Expected tissue specificity : Ubiquitous





The different images obtained by double labeling immunofluorescence microscopy of CAPAN- 2 (Pancreas adenocarcinoma) cell line shows that SMAD5 is a cytoplasmic and nuclear protein detected in this cellular tissue.

Pictures are compared with a negative control from the same cell line without primary Ab.

Remaining cell lines tested gave a positive result with a nuclear distribution.