

PRODUCT INFORMATION

Product name : WT1 antibody

Product type : Primary antibodies

Description : Mouse monoclonal to WT1

Immunogen : 1 synthetic peptide (human) conjugated to KLH

Reacts with : Hu, Ms

Tested applications : ELISA, WB & IF

GENE INFORMATION

Gene Symbol : WT1

Gene Name : Wilms tumor 1

Ensembl ID : ENSG00000184937

Entrez GeneID : 7490

Swiss-Prot : P19544

Molecular weight : 56.9, 55.2, 49.2, 48.9, 47.5, 47.2, 34.4, 33.1 kDa

Function : Transcription factor that plays an important role in cellular development and cell survival. Regulates the expression of numerous target genes, including EPO. Plays an essential role for development of the urogenital system. Recognizes and binds to the DNA sequence 5'-CGCCCCGC-3'. It has a tumor suppressor as well as an oncogenic role in tumor formation. Function may be isoform-specific: isoforms lacking the KTS motif may act as transcription factors. Isoforms containing the KTS motif may bind mRNA and play a role in mRNA metabolism or splicing. Isoform 1 has lower affinity for DNA, and can bind RNA.

Expected subcellular localization : Nucleus. Nucleus › nucleolus. Cytoplasm. Note: Shuttles between nucleus and cytoplasm.

Expected tissue specificity : Expressed in the kidney and a subset of hematopoietic cells

Summary : This gene encodes a transcription factor that contains four zinc-finger motifs at the C-terminus and a proline/glutamine-rich DNA-binding domain at the N-terminus. It has an essential role in the normal development of the urogenital system, and it is mutated in a small subset of patients with Wilm's tumors. This gene exhibits complex tissue-specific and polymorphic imprinting pattern, with biallelic, and monoallelic expression from the maternal and paternal alleles in different tissues. Multiple transcript variants have been described. In several variants, there is evidence for the use of a non-AUG (CUG) translation initiation site upstream of and in-frame with the first AUG. Authors of PMID:7926762 also provide evidence that WT1 mRNA undergoes RNA editing in human and rat, and that this process is tissue-restricted and developmentally regulated. [provided by RefSeq, Oct 2010]

APPLICATION NOTE

Recommended dilution :

- **ELISA:** Antibody specificity was verified by direct ELISA against the 1 immunogen peptide. A titer of 233000 has been determined. Appropriate specificity controls were run.
- **WB:** Dilution 1/5000
- **IF:** Dilution 1/50

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

Raised in : Mouse

Clonality : Monoclonal

Isotype : IgG

Purity : Purified Antibody

Concentration: 2mg/ml

Storage buffer : Containing a final concentration of PBS/glycerol (V/V), 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

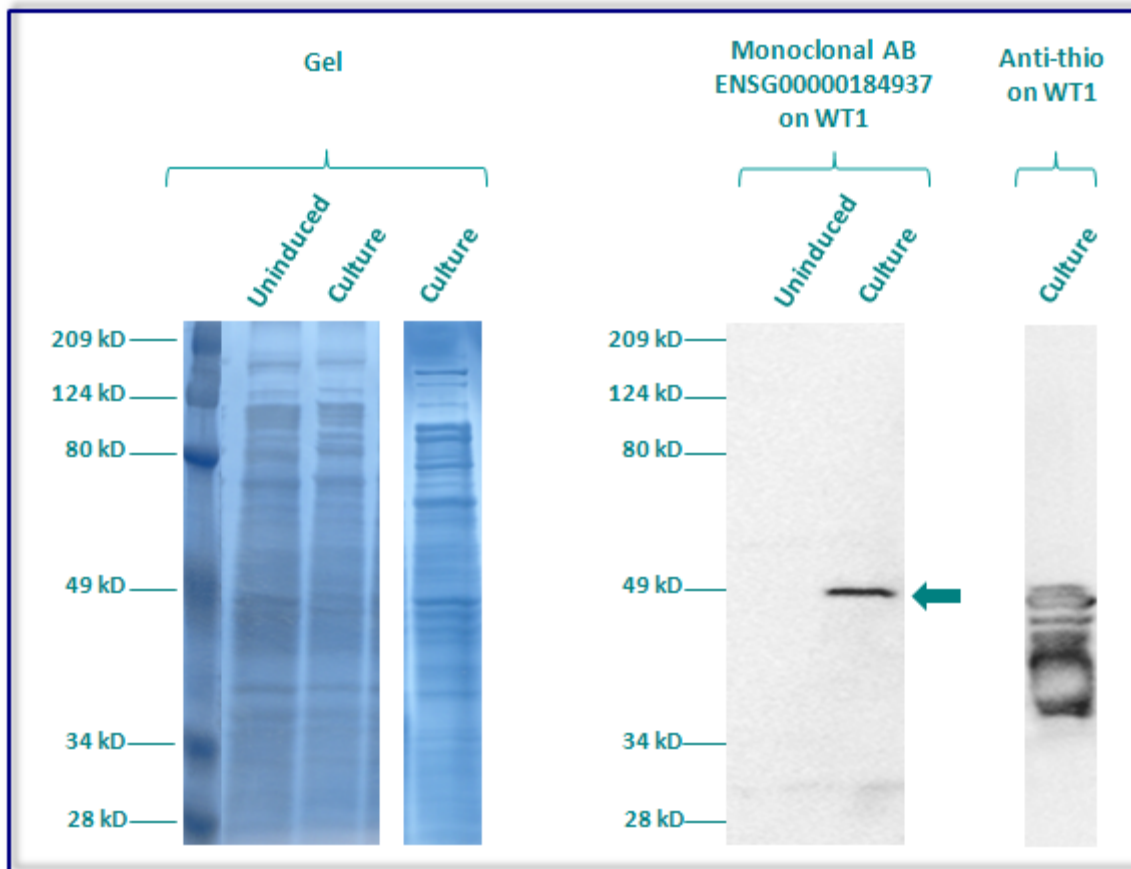
The monoclonal purified antibody ENSG00000184937 has tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

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

Clone : 5C12F12F8, Isotype : G1; kappa

Plasmid name : pBAD-DEST49.

Molecular weight of WT1 : 48.4kDa (34.4kDa + 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 ENSG00000184937 at 1:5000
- Dilute the anti-thio at 1:5000

60 minutes of incubation

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

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

IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescence analysis of Wilms tumor protein (WT1) expression in 6 cells lines (HELA, 293T/17, Capan-2, SAOS-2, SH-SY5Y, Skin 3,44). The monoclonal antibody ENSG00000184937 has been tested at 1/50.

Green staining : cytoskeleton (microtubules/ α -tubuline)

Blue staining : nucleus (Hoechst)

Red staining : anti- WT1 antibody (purified)

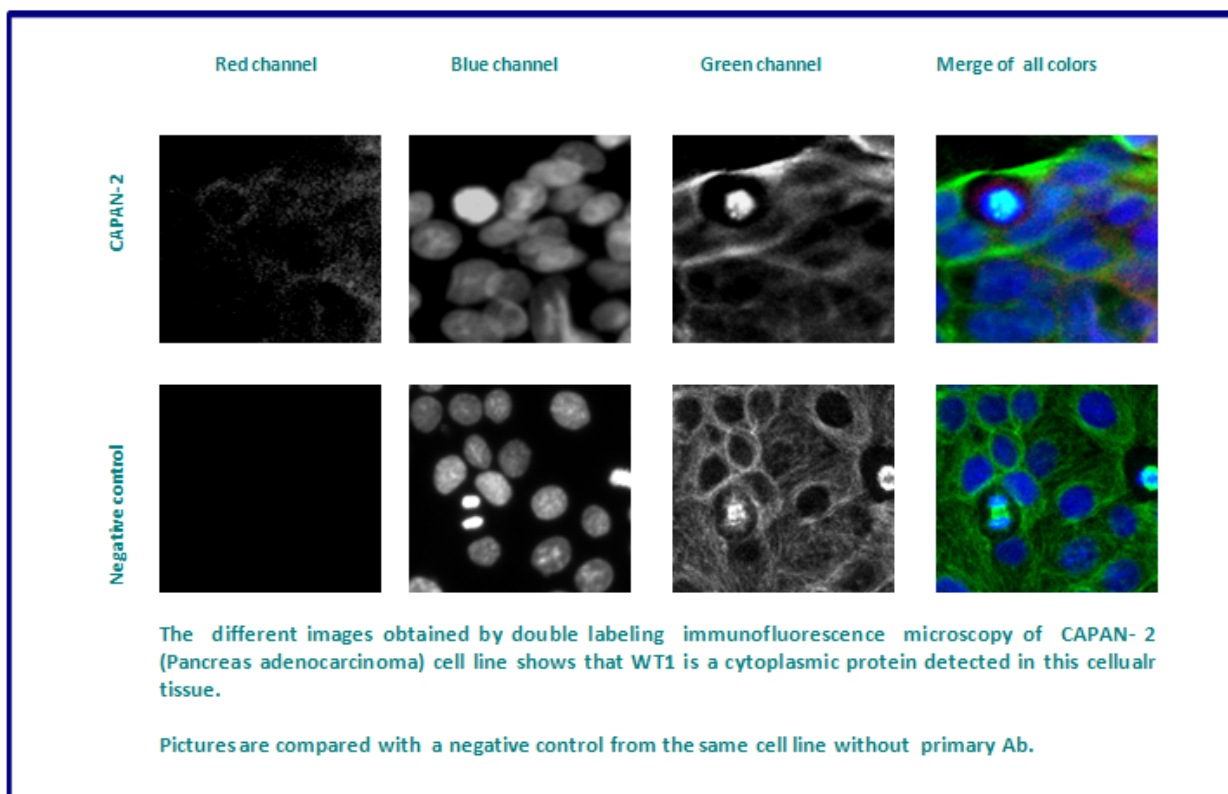
Expected subcellular location : Nucleus. Cytoplasm.

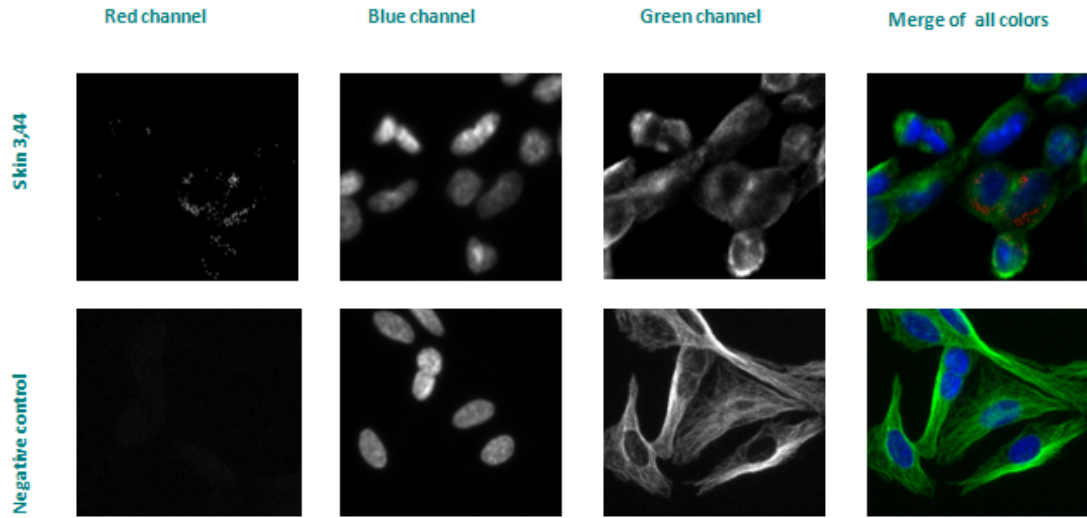
Note: Shuttles between nucleus and cytoplasm.

Isoform 1: Nucleus speckle

Isoform 4: Nucleus > nucleoplasm

Expected tissue specificity : Expressed in the kidney and a subset of hematopoietic cells





The different images obtained by double labeling immunofluorescence microscopy of Skin 3,44 (melanoma) cell line shows that WT1 is a cytoplasmic 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 negative result under these conditions.