abcam

ab216646

Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	CAN-R9(IHC)-56-2
Purification technique	Affinity purification Protein A
Specificity	Expression levels of the target protein vary with sample type and some optimisation may be required.
Concentration	0.963 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Not recommended

Species	Mouse, Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information WT1

Function	Transcription factor that plays an important role in cellular development and cell survival (PubMed:7862533). Recognizes and binds to the DNA sequence 5'-GCG(T/G)GGGCG-3' (PubMed:17716689, PubMed:25258363, PubMed:7862533). Regulates the expression of numerous target genes, including EPO. Plays an essential role for development of the urogenital system. 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 (PubMed:15520190). Isoforms containing the KTS motif may bind mRNA and play a role in mRNA metabolism or splicing (PubMed:16934801). Isoform 1 has lower affinity for DNA, and can bind RNA (PubMed:19123921).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab216646 is the carrier-free version of ab89901.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The Wilms Tumor Protein commonly known as WT1 is a critical transcription factor implicated in several cellular processes. It is also identified by other names such as Wilms Tumor Suppressor and WT33. WT1 protein has a mass of approximately 52-54 kDa. It is broadly expressed in various tissues with high levels in the developing kidney gonads and certain mesothelial tissues. Its expression plays an important role in organ development and cellular differentiation.
Biological function summary	The WT1 protein regulates gene expression by binding to specific DNA sequences influencing cell growth and differentiation. WT1 acts as a part of larger protein complexes interacting with other transcription factors and co-regulators. It plays essential roles in the development of the urogenital system and in the

	maintenance of mesothelial cells. The WT1 protein is essential for normal kidney and gonadal development highlighting its significance in embryogenesis.
Pathways	WT1 integration involves multiple cellular signaling cascades. It is notably engaged in the Wnt and PI3K/AKT signaling pathways which are critical for cell proliferation and survival. In these pathways WT1 interaction with proteins such as β-catenin and PI3K subunits modulates cell fate decisions. This involvement in signaling networks exemplifies its importance in cellular homeostasis and response to environmental cues.
Associated diseases and disorders	WT1 mutations or dysregulation associate strongly with specific pathologies particularly Wilms tumor and acute myeloid leukemia (AML). In Wilms tumor WT1 acts as a tumor suppressor gene and its loss of function leads to tumorigenesis in the kidney. In AML aberrant WT1 expression affects normal hematopoiesis and is often linked to poor prognosis. Interaction with proteins like BSA in immunohistochemistry diagnostics highlights WT1's significance as a biomarker for these diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

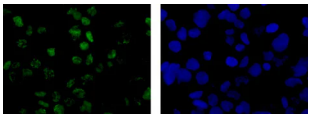
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

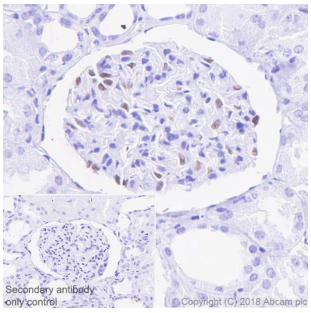
11 product images



Immunocytochemistry/ Immunofluorescence - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Wilms Tumor Protein antibody

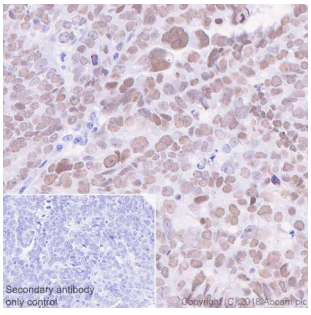
Immunocytochemsitry/Immunofluorescence analysis of K562 cells labelling Wilms Tumor Protein (green) with purified ab89901 at 1/50. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Wilms Tumor Protein antibody

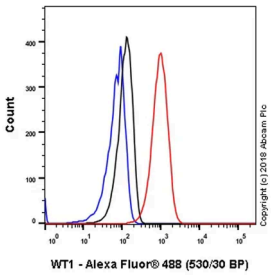
ab89901 staining Wilms Tumor Protein in paraffin-embedded Human kidney tissue sections by Immunohistochemistry. Antigen retrieval was by heat mediation using citrate buffer (pH 6.0). Samples were incubated with primary antibody at 1:500 dilution (0.49 µg/ml). A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Nuclear staining on human kidney glomerulus (PMID: 12898605).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Wilms Tumor Protein antibody

ab89901 staining Wilms Tumor Protein in paraffin-embedded Human ovarian serous adenocarcinoma tissue sections by Immunohistochemistry. Antigen retrieval was by heat mediation using citrate buffer (pH 6.0). Samples were incubated with primary antibody at 1:500 dilution (0.49 µg/ml). A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Nuclear staining on human ovarian serous adenocarcinoma (PMID: 11939727).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).

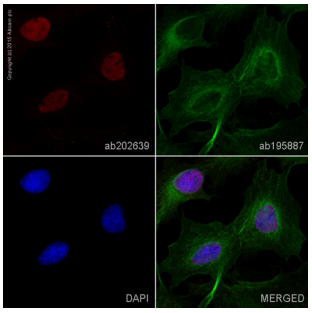


Flow Cytometry (Intracellular) - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Flow Cytometry (Intracellular) staining using rabbit Anti-Wilms Tumor Protein antibody

Intracellular Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labeling Wilms Tumor Protein with ab89901 at 1/200 dilution (0.1µg)/ Red. Cells

were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (ab172730) / Black was used as the isotype control. Cells without incubation with primary antibody and secondary antibody / Blue was used as the unlabeled control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).

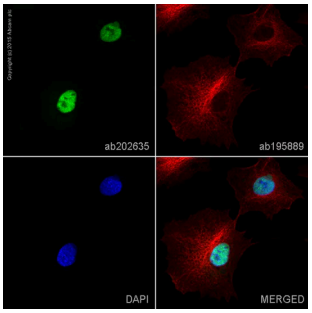


Immunocytochemistry/ Immunofluorescence - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Wilms Tumor Protein antibody

Clone CAN-R9(IHC)-56-2 (ab216646) has been successfully conjugated by Abcam. This image was generated using Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] (Alexa Fluor® 647). Please refer to ab202639 for protocol details.
ab202639 staining Wilms Tumor Protein in HepG2 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab202639 at 1/200 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



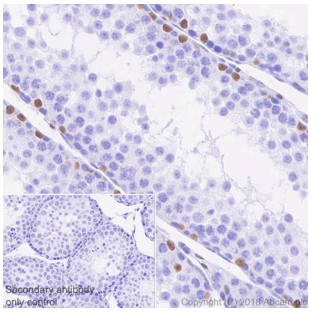
Immunocytochemistry/ Immunofluorescence - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Wilms Tumor Protein antibody

Clone CAN-R9(IHC)-56-2 (ab216646) has been successfully conjugated by Abcam. This image was generated using Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] (Alexa Fluor® 488). Please refer to ab202635 for protocol details.
ab202635 staining Wilms Tumor Protein in HepG2 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab202635 at 1/200 dilution (shown in green) and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HepG2 cells fixed with 100% methanol (5min).

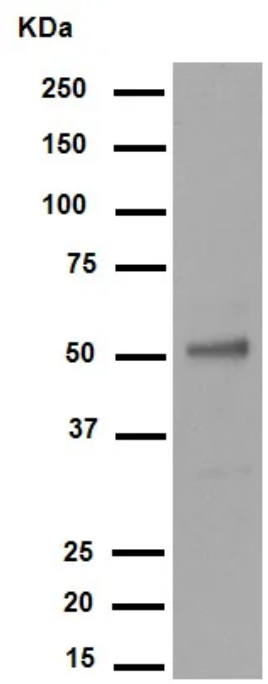


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Wilms Tumor Protein antibody

ab89901 staining Wilms Tumor Protein in paraffin-embedded Mouse testis tissue sections by Immunohistochemistry.
Antigen retrieval was by heat mediation using citrate buffer (pH 6.0). Samples were incubated with primary antibody at 1:500 dilution (0.49 µg/ml). A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Nuclear staining on Sertoli cells in mouse testis (PMID: 21863216).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).



Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Western blot staining of K562 (Human chronic myelogenous leukemia cell line from bone marrow) cell lysate using rabbit Anti-Wilms Tumor Protein antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).
Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] (ab89901) at 1/1000 dilution

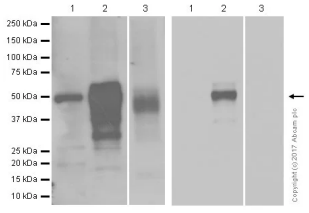
All lanes:
K562 (Human chronic myelogenous leukemia cell line from bone marrow) cell lysate at 10 µg

Secondary

All lanes:
Peroxidase-conjugated goat anto-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 49 kDa

Observed band size: 55 kDa



Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Western blot staining using rabbit Anti-Wilms Tumor Protein antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).
Exposure time: Left image: 3 minutes
Right image: 15 seconds

This antibody shows low affinity in mouse sample.

Blocking and diluting buffer: 5% NFDM/TBST

All lanes:

Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] (ab89901) at 1/500 dilution

Lane 1:
Human testis whole cell lysate. at 15 µg

Lane 2:
K562 (Human T cell leukemia T lymphocyte) whole cell lysates at 4 µg

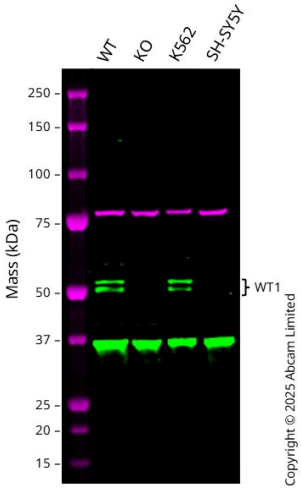
Lane 3:
Mouse testis whole cell lysates at 15 µg

Secondary

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

Predicted band size: 49 kDa

Observed band size: 55 kDa



Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Western blot staining using rabbit Anti-Wilms Tumor Protein antibody

This data was developed using ab89901, the same antibody clone in a different buffer formulation. Western blot: Rabbit Monoclonal [CAN-R9(IHC)-56-2] to Wilms Tumor Protein ab89901 staining at 1/500 dilution, shown in green; Mouse anti-CANX (ab238078) loading control staining at 1/20,000 dilution, shown in magenta.

A band was observed at 50/61 kDa in Wild-type HAP1 cell lysates with no signal observed at this size in WT1 knockout HAP1 cell line.

To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.

Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] (ab89901) at 1/500 dilution

Lane 1:
Wild-type HAP1 at 20 µg

Lane 2:
WT1 knockout HAP1 at 20 µg

Lane 3:
K562 at 20 µg

Lane 4:
SH-SY5Y at 20 µg

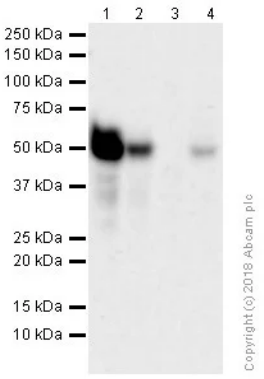
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 61 kDa



Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] - BSA and Azide free (ab216646)

Wilms Tumor Protein Western blot staining using rabbit Anti-Wilms Tumor Protein antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab89901).
WT1 expression varies in different cell lines.

Blocking and diluting buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-Wilms Tumor Protein antibody [CAN-R9(IHC)-56-2] (ab89901) at 1/5000 dilution

Lane 1:
K-562 (Human chronic myelogenous leukemia lymphoblast). Whole cell lysates at 20 µg

Lane 2:
THP-1 (Human monocytic leukemia monocyte). Whole cell lysates at 20 µg

Lane 3:
SH-SY5Y (Human neuroblastoma epithelial cell) Whole cell lysates at 20 µg

Lane 4:
HeLa (Human cervix adenocarcinoma epithelial cell) Whole cell lysates at 20 µg

Secondary

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

Predicted band size: 49 kDa

Exposure time: 3min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.