abcam

Product datasheet

Anti-WSTF antibody [EP1704Y] - BSA and Azide free ab235388

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-WSTF antibody [EP1704Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1704Y] to WSTF - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, 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: Wild-type HAP1, HeLa (**ab150035**), and PC12 cell lysates. Mouse and Rat testis. B16-F0, MCF7, HeLa whole cell lysates. Flow cyt: HeLa cells; IHC: rat cardiac muscle tissue, mouse cardiac muscle tissue, human breast carcinoma; ICC/IF: HeLa cells.
General notes	ab235388 is the carrier-free version of **ab51256**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1704Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab235388 in the following tested applications.

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

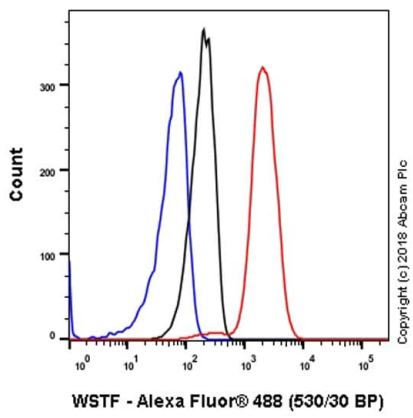
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 185 kDa (predicted molecular weight: 171 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

Target

Function Atypical tyrosine-protein kinase that plays a central role in chromatin remodeling and acts as a transcription regulator. Involved in DNA damage response by phosphorylating 'Tyr-142' of histone H2AX (H2AXY142ph). H2AXY142ph plays a central role in DNA repair and acts as a mark that distinguishes between apoptotic and repair responses to genotoxic stress. Essential component of the WICH complex, a chromatin remodeling complex that mobilizes nucleosomes and reconfigures irregular chromatin to a regular nucleosomal array structure. The WICH complex regulates the transcription of various genes, has a role in RNA polymerase I and RNA polymerase III transcription, mediates the histone H2AX phosphorylation at 'Tyr-142', and is involved in the maintenance of chromatin structures during DNA replication processes. In the complex, it mediates the recruitment of the WICH complex to replication foci during DNA replication. Also involved in vitamin D-coupled transcription regulation via its association with the WINAC complex, a chromatin-remodeling complex recruited by vitamin D receptor (VDR), which is required for the

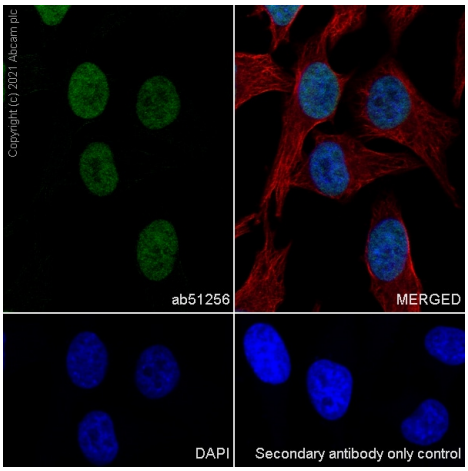
	ligand-bound VDR-mediated transrepression of the CYP27B1 gene. In the WINAC complex, plays an essential role by targeting the complex to acetylated histones, an essential step for VDR-promoter association.
Tissue specificity	Ubiquitously expressed with high levels of expression in heart, brain, placenta, skeletal muscle and ovary.
Involvement in disease	Note=BAZ1B is located in the Williams-Beuren syndrome (WBS) critical region. WBS results from a hemizygous deletion of several genes on chromosome 7q11.23, thought to arise as a consequence of unequal crossing over between highly homologous low-copy repeat sequences flanking the deleted region. Haploinsufficiency of BAZ1B may be the cause of certain cardiovascular and musculo-skeletal abnormalities observed in the disease.
Sequence similarities	Belongs to the WAL family. BAZ1B subfamily. Contains 1 bromo domain. Contains 1 DDT domain. Contains 1 PHD-type zinc finger. Contains 1 WAC domain.
Developmental stage	Expressed at equal levels in 19-23 weeks old fetal tissues.
Domain	The N-terminal part (1-345), including the WAC domain and the C motif, mediates the tyrosine-protein kinase activity. The bromo domain mediates the specific interaction with acetylated histones.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR.
Cellular localization	Nucleus. Accumulates in pericentromeric heterochromatin during replication. Targeted to replication foci throughout S phase via its association with PCNA.

Images



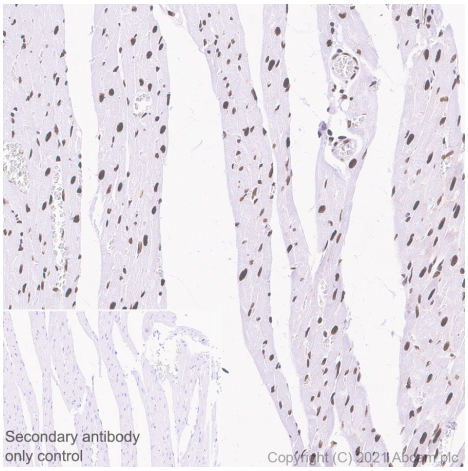
Flow Cytometry (Intracellular) - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

This data was developed using **ab51256**, the same antibody clone in a different buffer formulation.
Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling WSTF with Purified **ab51256** at 1:20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



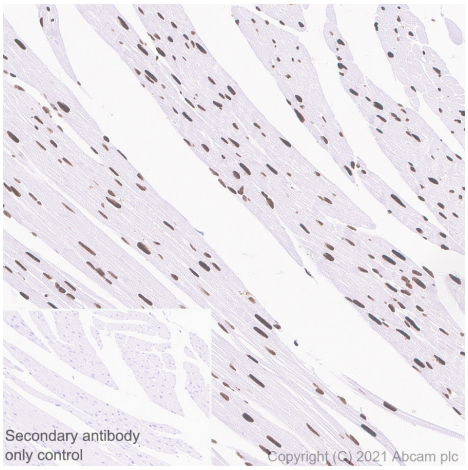
Immunocytochemistry/ Immunofluorescence - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

This data was developed using **ab51256**, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling WSTF with Purified **ab51256** at 1:50 dilution (2.9 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



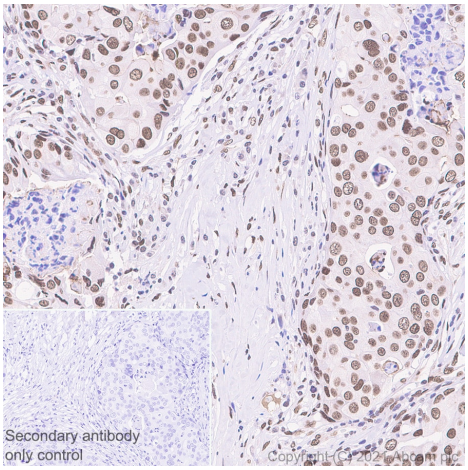
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

This data was developed using **ab51256**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cardiac muscle tissue sections labeling WSTF with Purified **ab51256** at 1:700 dilution (0.211 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using **ab93678** (citrate buffer, pH 6.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



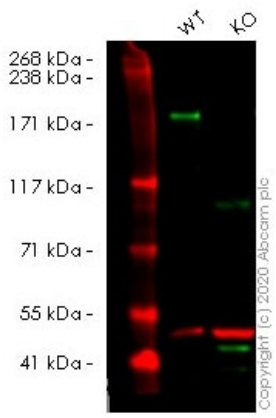
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

This data was developed using **ab51256**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cardiac muscle tissue sections labeling WSTF with Purified **ab51256** at 1:700 dilution (0.211 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using **ab93678** (citrate buffer, pH 6.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

This data was developed using **ab51256**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling WSTF with Purified **ab51256** at 1:700 dilution (0.211 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using **ab93678** (citrate buffer, pH 6.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

All lanes : Anti-WSTF antibody [EP1704Y] (**ab51256**) at 1/15000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : BAZ1B knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

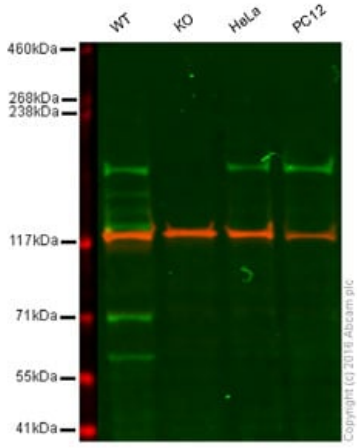
Performed under reducing conditions.

Predicted band size: 171 kDa
Observed band size: 175 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab51256**).

Lanes 1- 2: Merged signal (red and green). Green - **ab51256** observed at 175 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab51256 was shown to react with WSTF in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264907** (knockout cell lysate **ab257370**) was used. Wild-type HeLa and BAZ1B knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab51256** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 15000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

All lanes : Anti-WSTF antibody [EP1704Y] (**ab51256**) at 1/15000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : WSTF knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : PC12 cell lysate

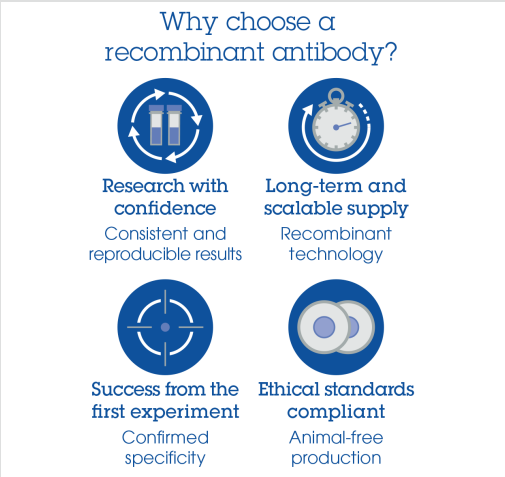
Lysates/proteins at 20 µg per lane.

Predicted band size: 171 kDa

This WB data was generated using the same anti-WSTF antibody clone, EP1704Y, in a different buffer formulation (cat# **ab51256**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab51256** observed at 175 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab51265 was shown to recognize WSTF in wilt-type cells along with additional cross-reactive bands as signal was lost in WSTF knockout samples. Wild-type and WSTF knockout samples were subjected to SDS-PAGE. **ab51256** and **ab18058** (loading control to vinculin) were diluted 1/15 000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Anti-WSTF antibody [EP1704Y] - BSA and Azide free (ab235388)

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