abcam

Product datasheet

Anti-Villin antibody [SP145] - BSA and Azide free ab245749

Recombinant RabMAb

7 Images

Overview

Product name	Anti-Villin antibody [SP145] - BSA and Azide free
Description	Rabbit monoclonal [SP145] to Villin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), mIHC, IP, IHC-P, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Chicken, Cow, Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Human colon, small intestine and kidney tissues; HT-29 cell lysate. mIHC: Human colon tissue. Flow cyto (intra): HT-29 cells
General notes	ab245749 is the carrier-free version of **ab130751**. 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**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A/G purified
Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP145
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab245749 in the following tested applications.

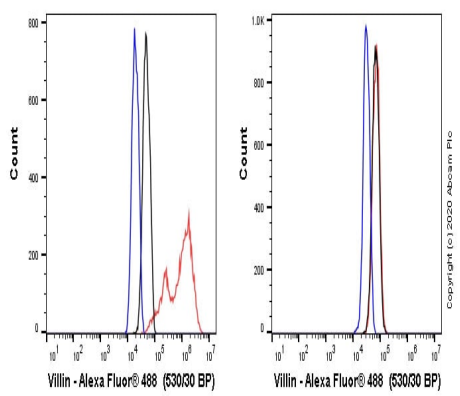
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
mIHC		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Primary incubation for 10 minutes at room temperature.
WB		Use at an assay dependent concentration. Predicted molecular weight: 93 kDa. Incubate for 1 hour at room temperature.

Target

Function	Ca(2+)-regulated actin-binding protein.
Tissue specificity	Major component of microvilli of intestinal epithelial cells and kidney proximal tubule cells.
Sequence similarities	Belongs to the villin/gelsolin family. Contains 6 gelsolin-like repeats. Contains 1 HP (headpiece) domain.
Domain	Consists of a large core fragment, the N-terminal portion, and a small headpiece, the C-terminal portion. The headpiece binds F-actin strongly in both the presence and absence of calcium.
Cellular localization	Cytoplasm > cytoskeleton.

Images



Flow Cytometry (Intracellular) - Anti-Villin antibody [SP145] - BSA and Azide free (ab245749)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab130751**).

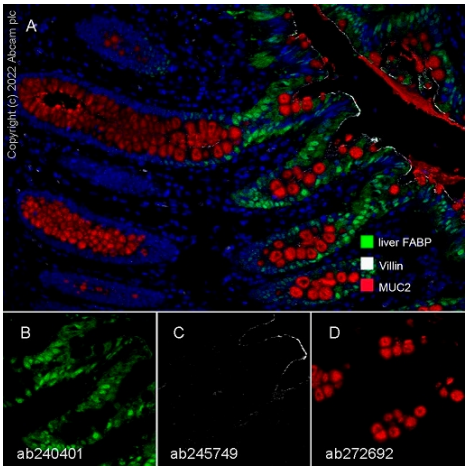
Flow cytometry overlay histogram showing left HT-29 positive cells and right negative PANC-1 stained with **ab130751** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab130751**) (1x 10^6 in 100µl at 0.2µg/ml (1/11200)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

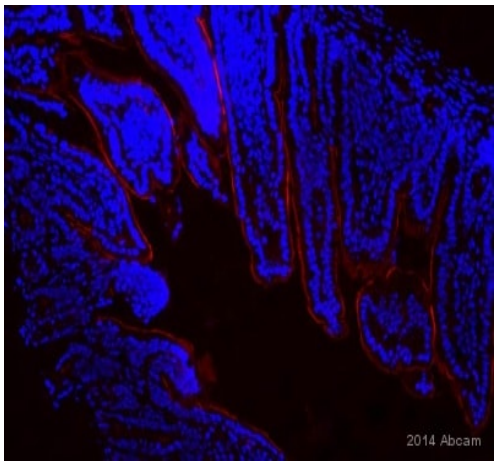
This antibody gave a positive signal in HT-29 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Multiplex immunohistochemistry - Anti-Villin antibody [SP145] - BSA and Azide free (ab245749)

Fluorescence multiplex immunohistochemical analysis of the human colon (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-Villin (ab245749, gray; Opal™690), anti-liver FABP (**ab240401**, green; Opal™520) and anti-MUC2 (**ab272692**, red; Opal™570) on human colon. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-Villin stained on apical border. Panel D: anti-MUC2 stained on goblet cells. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab245749 (1/1000 dilution), **ab240401** (1/8000 dilution), and **ab272692** (1/5000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

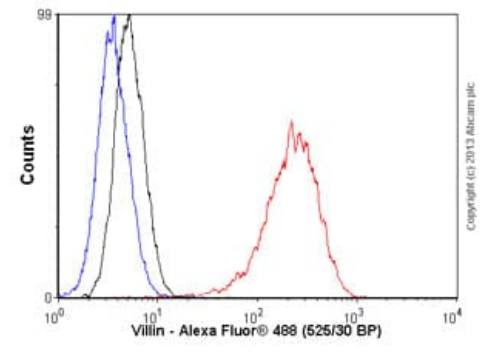
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab130751**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Villin antibody [SP145] - BSA and Azide free (ab245749)

ab130751 staining Villin in mouse intestine tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and blocked with 5% serum for 30 minutes at 25°C; antigen retrieval was by heat mediation in 10mM citrate buffer, pH 6. Samples were incubated with primary antibody (1/1000) for 16 hours at 4°C. An Alexa Fluor® 568-conjugated goat anti-rabbit IgG monoclonal (1/250) was used as the secondary antibody.

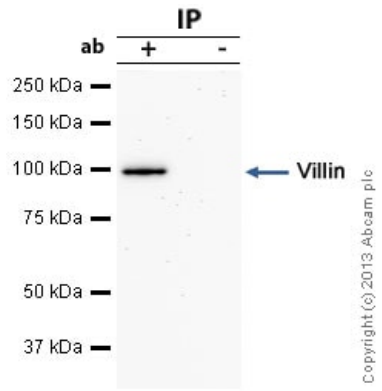
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab130751**).



Flow Cytometry (Intracellular) - Anti-Villin antibody [SP145] - BSA and Azide free (ab245749)

Overlay histogram showing Caco 2 cells stained with **ab130751** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab130751**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab130751**).



Immunoprecipitation - Anti-Villin antibody [SP145] - BSA and Azide free (ab245749)

Villin was immunoprecipitated using 0.5mg SW480 whole cell extract, 5µg of Rabbit polyclonal to Villin and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

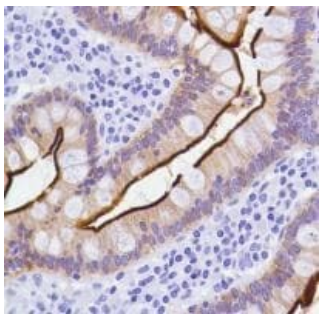
The antibody was incubated under agitation with Protein G beads for 10min, SW480 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with **ab130751**.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 93kDa; Villin

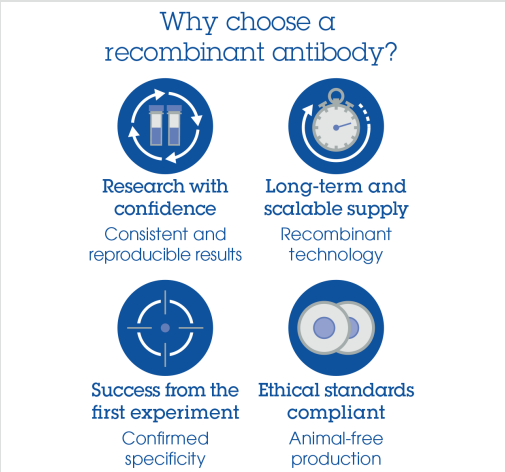
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab130751**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Villin antibody [SP145] - BSA and Azide free (ab245749)

ab130751, at 1/100 dilution, staining Villin in formalin-fixed, paraffin-embedded Human colon tissue by Immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab130751**).



Anti-Villin antibody [SP145] - BSA and Azide free

(ab245749)

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