Antibody Validation Report

aVR.VIM.P08670.AB_306907.v1.0 (March 25_2021)

A. Basic Target Information

Target Information

UniProt Accession Number: P08670

Target Name: Vimentin

Antibody Information

RRID: AB_306907

Antibody Name: Anti-Vimentin antibody [RV202]
Host Organism: Mouse

Clonality: Monoclonal
Vendor: AbCam
Catalog Number: ab8978
Lot Number: GR3361308-1
Recombinant (Y/N): No
Organ/Tissue used for validation: Kidney
HuBMAP Platform Used: IP-MS

Protocols.io doi for Validation Protocol: 10.17504/protocols.io.btzvnp66
ORCID ID of submitter: 0000-0002-4745-8129

B. Validation Data

B.1. Vendor Validation: WB, IF, IHC, IHC(P), FC

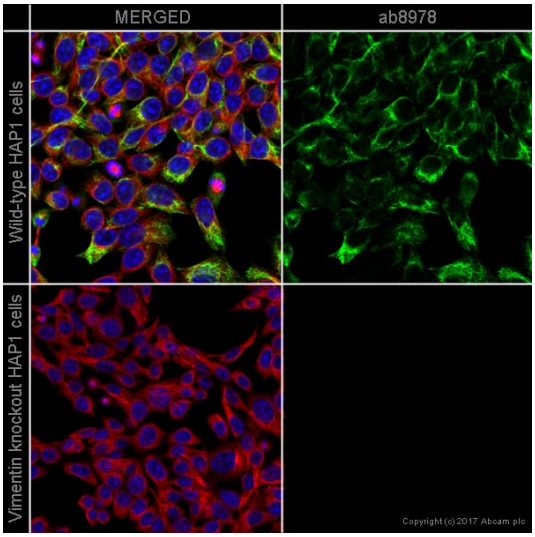
All lanes : Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

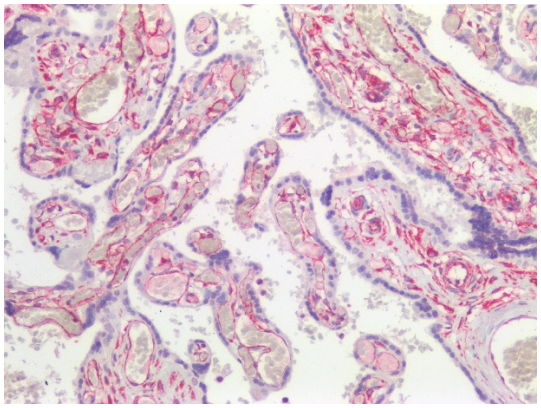
Lane 1 : NIH3T3 cell lysate
Lane 2 : Normal human dermal fibroblasts cell extracts.

Predicted band size: 53 kDa
Observed band size: 57 kDa

Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

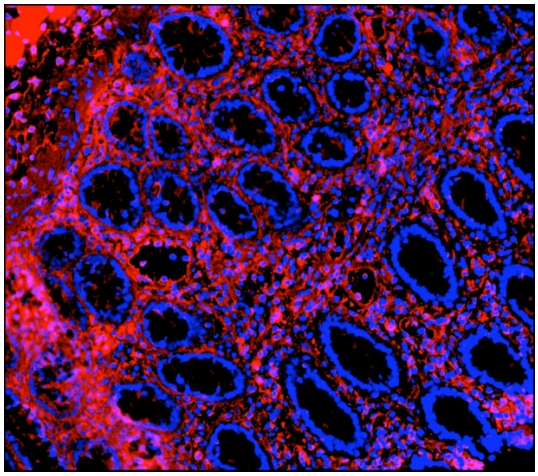
ab8978 staining Vimentin in wild-type HAP1 cells (top panel) and VIM knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab8978 at 1µg/ml and ab202272 (Rabbit monoclonal [EP1332Y] to alpha Tubulin (Alexa Fluor® 594)) at 1/250 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with ab150117 (Goat secondary antibody to Mouse IgG (Alexa Fluor® 488)) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.





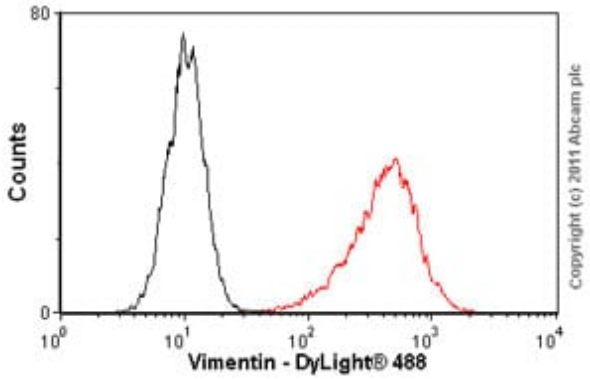
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Immunohistochemistry analysis of formalin fixed, paraffin embedded section of human placenta labeling Vimentin with ab8978, showing positive staining in connective tissue cells and no reactivity in epithelial cells.



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Immunohistochemistry analysis of frozen section of pig colon labeling Vimentin with ab8978, showing positive staining in connective tissue cells and no reactivity in epithelial cells. Nuclear staining with DAPI.



Date Accessed: 03/25/21

URL: https://www.abcam.com/vimentin-antibody-rv202-cytoskeleton-marker-ab8978.html

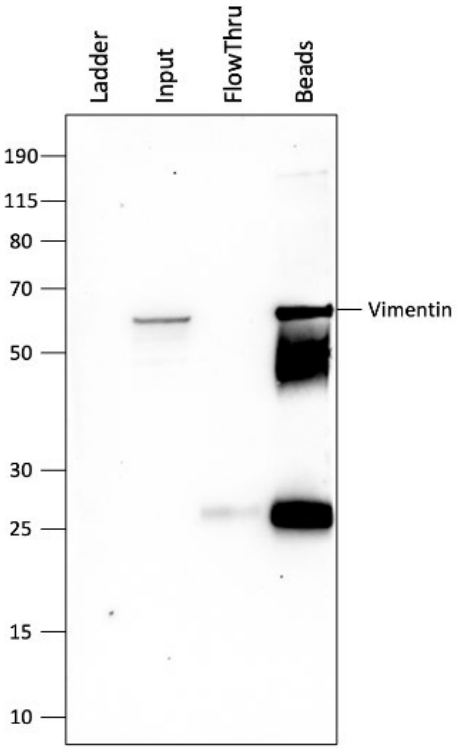
Flow Cytometry - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Overlay histogram showing HeLa cells stained with ab8978 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Triton 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 (ab8978, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This anti-Vimentin antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Triton used under the same conditions.

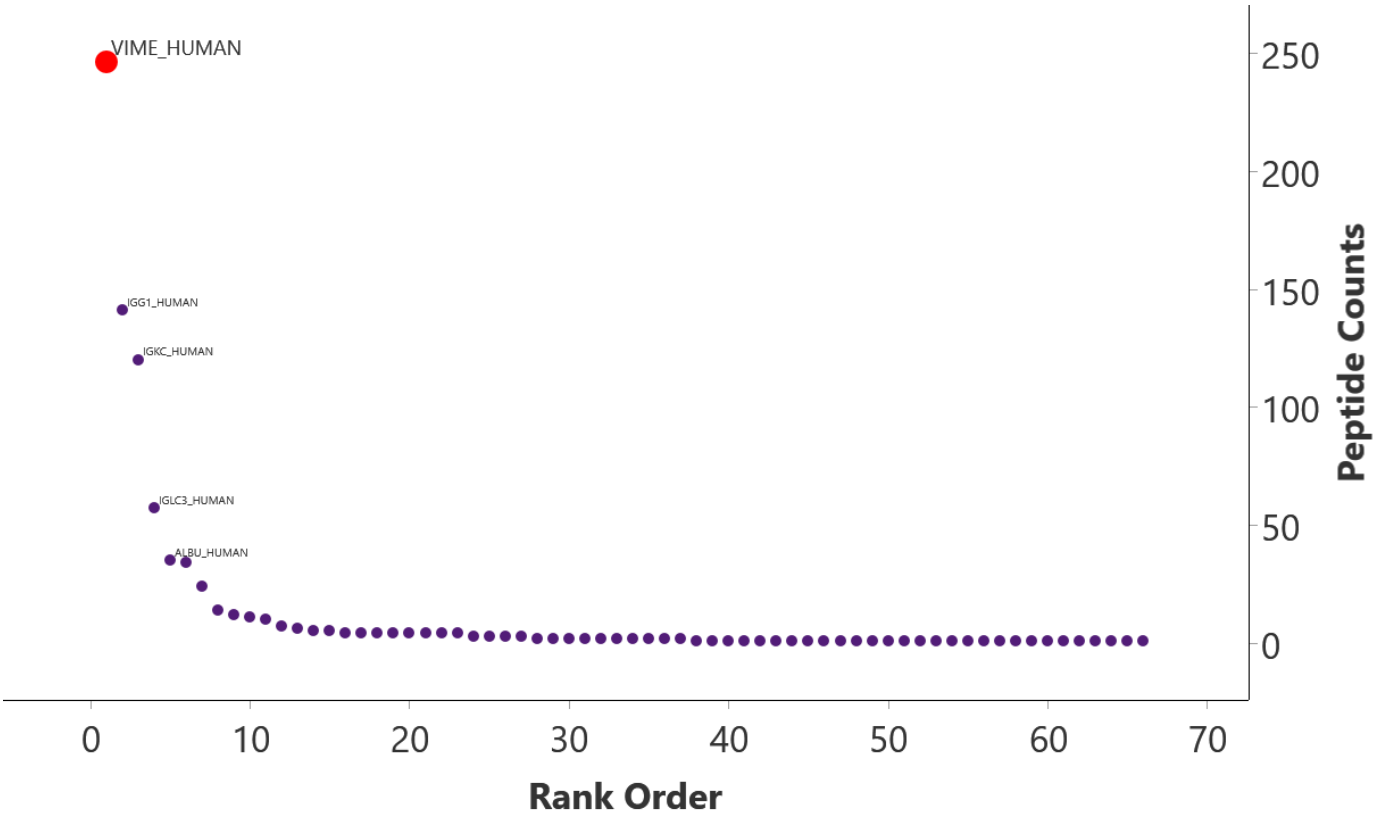
B.2. Laboratory Validation:

Immunoprecipitation/Western Blot

Vimentin
Mass: 53 kDa

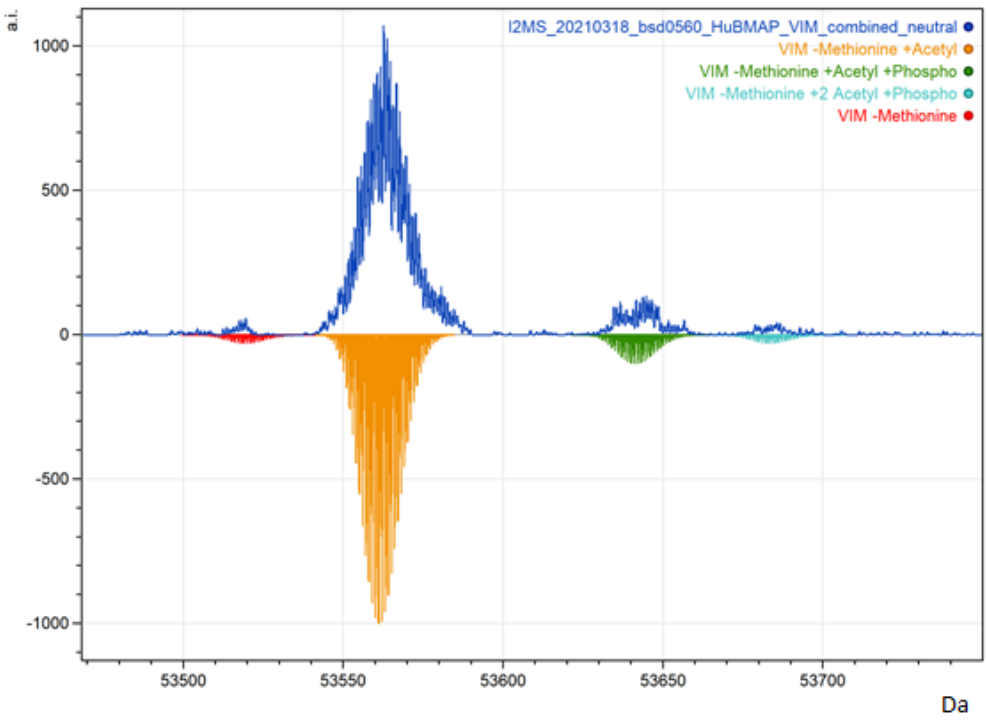


Immunoprecipitation/Bottom-Up MS



Immunoprecipitation/Top-Down MS

I2MS



Proteoforms Identified:

1: PFR00000001800, Vimentin, N-terminal Acetylation, 53,530.05 Da
[MOD:00394]-
STRSVSSSSYRRMFGGPGTASRPSSSRSYVTTSTRTYSLGSALRPSTSRSLYASSPGGVYAT
RSSAVRLRSSVPGVRLLQDSVDFSLADAINTEFKNTRTNEKVELQELNDRFANYIDKVRFLEQQ
NKILLAELEQLKGQGKSRLGDLYEEEMRELRRQVDQLTNDKARVEVERDNLAEDIMRLREKLQ
EEMLQREEAENTLQSFRQDVDNASLARLDLERKVESLQEEIAFLKKLHEEEIQELQAQIQEQHV
QIDVDVSKPDLTAALRDVRQQYESVAAKNLQEAEEWYKSKFADLSEAANRNNDALRQAKQES
TEYRRQVQSLTCEVDALKGTNESLERQMREMEENFAVEAANYQDTIGRLQDEIQNMKEEMAR
HLREYQDLLNVKMALDIEIATYRKLLEGEESRISLPLPNFSSLNLRETNLDSLPLVDTHSKRTLLIK
TVETRDGQVINETSQHHDDLE.

2: PFR00000003395, Vimentin, N-terminal Acetylation, T-Phosphorylation, 53,610.01 Da
[MOD:00394]-
STRSVSSSSYRRMFGGPGTASRPSSSRSYVTTSTRTYSLGSALRPSTSRSLYASSPGGVYAT
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NKILLAELEQLKGQGKSRLGDLYEEEMRELRRQVDQLTNDKARVEVERDNLAEDIMRLREKLQ
EEMLQREEAENTLQSFRQDVDNASLARLDLERKVESLQEEIAFLKKLHEEEIQELQAQIQEQHV
QIDVDVSKPDLTAALRDVRQQYESVAAKNLQEAEEWYKSKFADLSEAANRNNDALRQAKQES
TEYRRQVQSLTCEVDALKGTNESLERQMREMEENFAVEAANYQDTIGRLQDEIQNMKEEMAR
HLREYQDLLNVKMALDIEIATYRKLLEGEESRISLPLPNFSSLNLRETNLDSLPLVDTHSKRTLLIK
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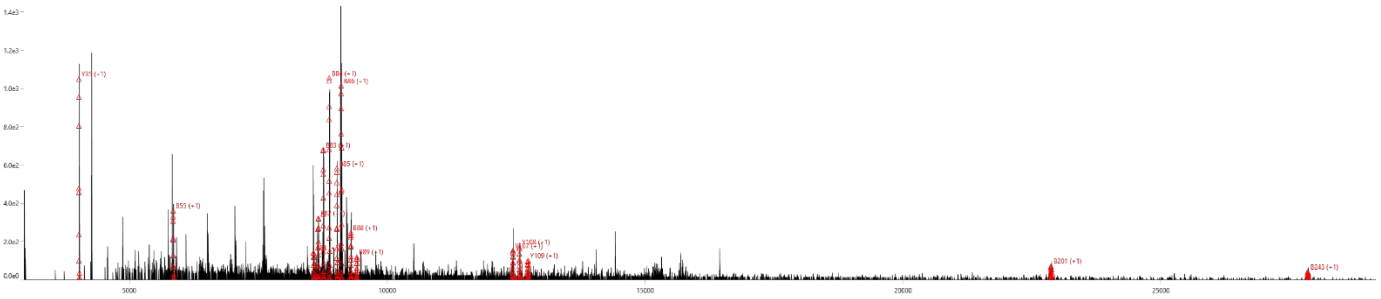
Intact Mass Profile



Protter (Omasits et al., Bioinformatics. 2013 Nov 21)