

## Product Datasheet

### Villin/VIL1 Rabbit Polyclonal Antibody (orb259634)

|                             |  |
|-----------------------------|--|
| <b>Catalog Number</b>       | orb259634  |
| <b>Category</b>             | Antibodies   |
| <b>Description</b>          | Villin/VIL1 Rabbit Polyclonal Antibody   |
| <b>Target</b>               | Villin-1   |
| <b>Clonality</b>            | Polyclonal   |
| <b>Species/Host</b>         | Rabbit   |
| <b>Isotype</b>              | Rabbit IgG   |
| <b>Conjugation</b>          | Unconjugated   |
| <b>Reactivity</b>           | Human, Mouse, Rat  |
| <b>Form/Appearance</b>      | Lyophilized  |
| <b>Concentration</b>        | Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.  |
| <b>Buffer/Preservatives</b> | Each vial contains antibody formulated with stabilizing components, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> , and 0.05 mg NaN <sub>3</sub> . *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required. |
| <b>Reconstitution</b>       | Add 0.2ml of distilled water will yield a concentration of 500ug/ml.   |
| <b>Purification</b>         | Immunogen affinity purified.   |
| <b>Immunogen</b>            | A synthetic peptide corresponding to a sequence at the C-terminus of human Villin, different from the related mouse sequence by three amino acids.   |

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

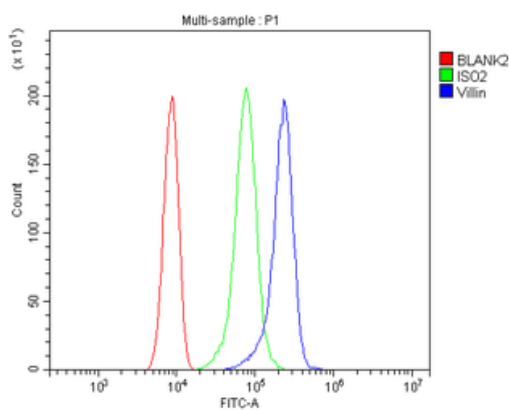
**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

|                            |  |
|----------------------------|--|
| <b>UniProt ID</b>          | <b>P09327</b>  |
| <b>MW</b>                  | 93 kDa   |
| <b>Tested applications</b> | FC, IF, IHC, WB  |
| <b>Dilution range</b>      | Western blot, 0.1-0.5µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml, Human, Mouse, Rat Immunofluorescence, 2µg/ml, Human, Mouse, Rat Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Human |
| <b>Specificity</b>         | No cross reactivity with other proteins.   |
| <b>Cross Reactivity</b>    | No cross-reactivity with other proteins  |
| <b>Antibody Type</b>       | Primary Antibody   |
| <b>Storage</b>             | Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.  |
| <b>Note</b>                | For research use only  |
| <b>Expiration Date</b>     | 12 months from date of receipt.  |



Flow Cytometry analysis of CACO-2 cells using anti-Villin antibody. Overlay histogram showing CACO-2 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Villin Antibody (1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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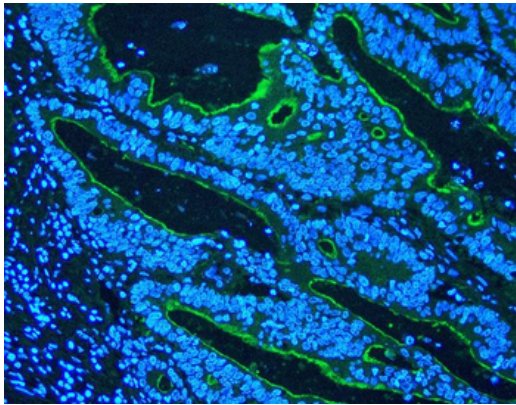
7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
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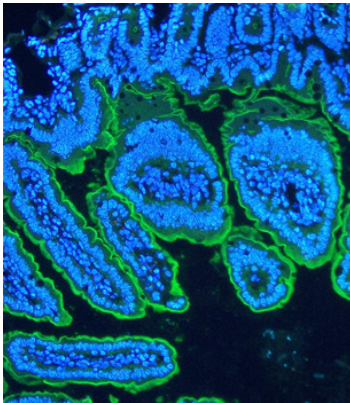
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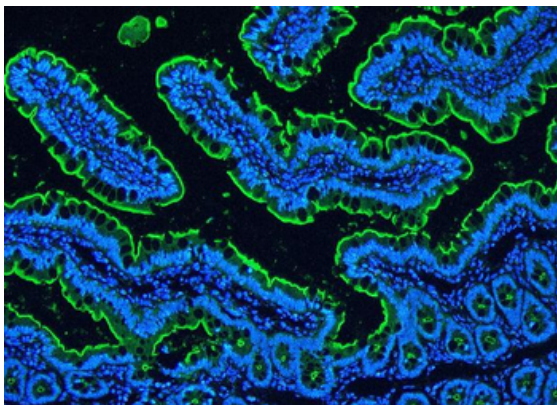
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IF analysis of Villi using anti-Villi antibody. Villi was detected in paraffin-embedded section of human rectal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu\text{g}/\text{mL}$  rabbit anti-Villi Antibody overnight at 4°C. DyLight488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of Villi using anti-Villi antibody. Villi was detected in paraffin-embedded section of mouse intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu\text{g}/\text{mL}$  rabbit anti-Villi Antibody overnight at 4°C. DyLight488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of Villi using anti-Villi antibody. Villi was detected in paraffin-embedded section of rat intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu\text{g}/\text{mL}$  rabbit anti-Villi Antibody overnight at 4°C. DyLight488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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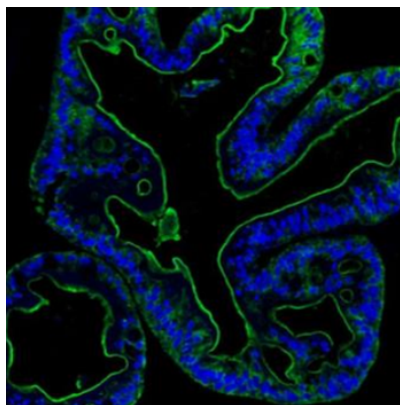
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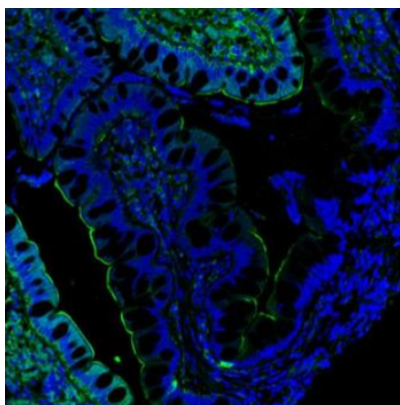
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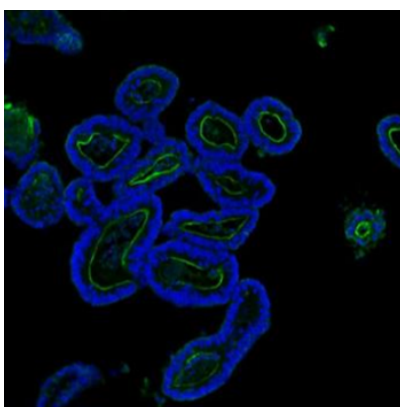
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IF analysis of Villin using anti-Villin antibody. Villin was detected in paraffin-embedded section of human colon organoid tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-Villin Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of Villin using anti-Villin antibody. Villin was detected in paraffin-embedded section of human ileum tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-Villin Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of Villin using anti-Villin antibody. Villin was detected in paraffin-embedded section of mouse ileum organoid tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-Villin Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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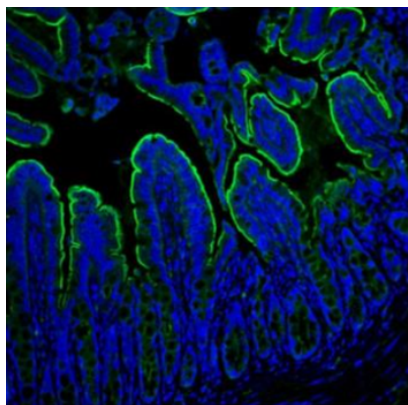
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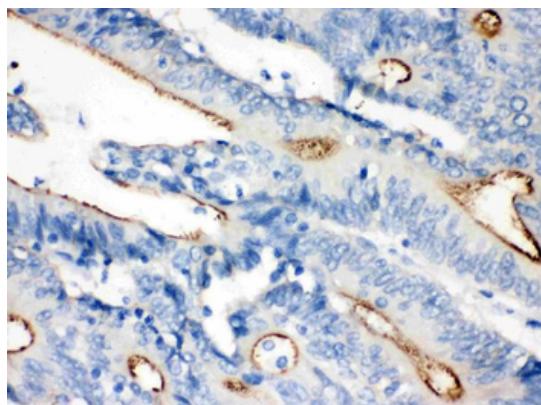
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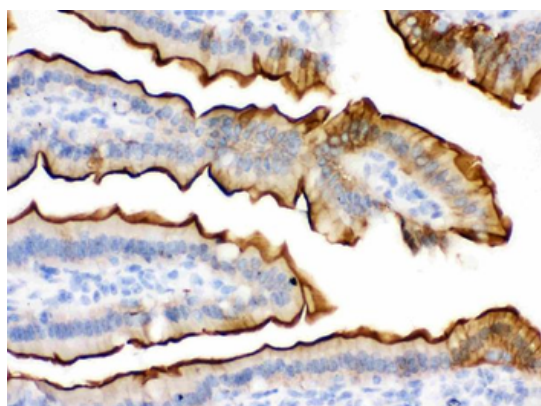
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IF analysis of Villin using anti-Villin antibody. Villin was detected in paraffin-embedded section of mouse ileum tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-Villin Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IHC analysis of Villin using anti-Villin antibody. Villin was detected in a paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml rabbit anti-Villin Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of Villin using anti-Villin antibody. Villin was detected in a paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml rabbit anti-Villin Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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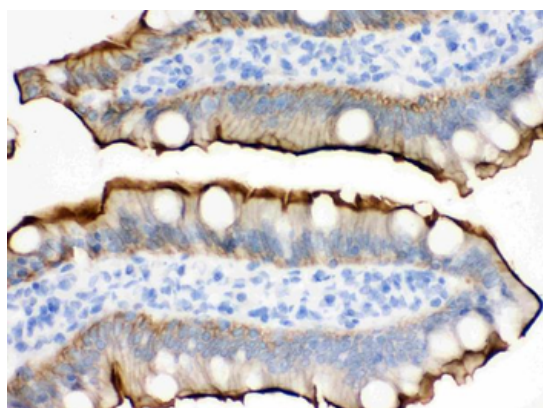
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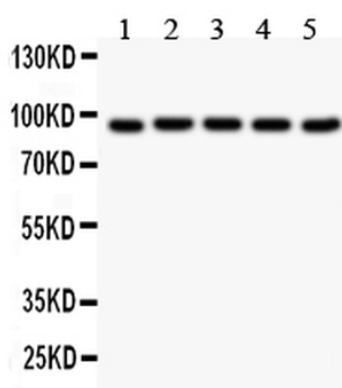
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IHC analysis of Villin using anti-Villin antibody. Villin was detected in a paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml rabbit anti-Villin Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



Western blot analysis of Villin using anti-Villin antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. Lane 1: Rat Intestine Tissue Lysate at 50  $\mu$ g. Lane 2: Mouse Kidney Tissue Lysate at 50  $\mu$ g. Lane 3: RH35 Whole Cell Lysate at 40  $\mu$ g. Lane 4: HEPG2 Whole Cell Lysate at 40  $\mu$ g, Lane 5: MCF-7 Whole Cell Lysate at 40  $\mu$ g. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Villin antigen affinity purified polyclonal antibody at 0.5  $\mu$ g/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for Villin at approximately 93 kDa. The expected band size for Villin is at 93 kDa.

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United Kingdom

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