

# Novocastra™ Liquid Mouse Monoclonal Antibody Villin

## Product Code: NCL-L-VILLIN

<b>Intended Use</b>	FOR RESEARCH USE ONLY.
<b>Specificity</b>	Human villin protein.
<b>Clone</b>	CWWB1
<b>Ig Class</b>	IgG1, kappa
<b>Antigen Used for Immunizations</b>	Prokaryotic recombinant protein corresponding to the C-terminal "headpiece" region of the human villin molecule.
<b>Hybridoma Partner</b>	Mouse myeloma (p3-NS1-Ag4-1).
<b>Preparation</b>	Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label.
<b>Effective on Frozen Tissue</b>	Yes. Acetone fixation recommended.
<b>Effective on Paraffin Wax Embedded Tissue</b>	Yes (using the high temperature antigen unmasking technique: see overleaf).
<b>Recommendations on Use</b>	Immunohistochemistry: Typical working dilution 1:100–1:200. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:500–1:1000.
<b>Positive Controls</b>	Immunohistochemistry: Small bowel. Western Blotting: CaCo2 cell line.
<b>Staining Pattern</b>	Cytoplasmic and membrane.
<b>Storage and Stability</b>	Store liquid antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Prepare working dilutions on the day of use.

### General Overview

Villin and the structurally-related proteins gelsolin, fragmin and severin, all regulate the framework and assembly of actin. Villin is composed of three domains. The first two domains are homologous and the third domain is called the "headpiece". This "headpiece" region is located at the C-terminus. Villin is unique among these proteins in its ability to cross-link actin filaments into bundles, a process observed only at low  $Ca^{2+}$  concentration. Villin is mainly produced by epithelial cells that develop a brush border. Cells producing villin are found either in the epithelial cells of the intestinal mucosa and gall bladder, or in epithelial cells of the kidney proximal tubules and ductuli efferentes of the testis. However, villin may also be found in some epithelia which lack a brush border but which are derived from embryonic gut such as duct cells of the exocrine pancreas and biliary cells of the liver. In these cell types, villin is concentrated in the apical cytoplasm. Epithelial cells of the intestinal mucosa are continually being renewed and this involves a migration of these cell types from the intestinal crypts to the tips of the villi, gradually acquiring their differentiated phenotype as they do so. The maximum production of villin occurs at the base of the villus. Villin, therefore, shows tissue-specific expression being restricted to certain epithelia and their apical domains, thus indicating their polarity.

### General References

- Toyoshima K, Seta Y, Takeda S, et al.. The Journal of Histochemistry and Cytochemistry. 46 (11): 1329–1334 (1998).
- Friederich E, Pringault E, Arpin M, et al.. BioEssays. 12 (9): 403–408 (1990).
- Janney P A and Matsudaira P T. The Journal of Biological Chemistry. 263 (32): 16738–16743 (1988).
- West A B, Isaac C A, Carboni J M, et al.. Gastroenterology. 94: 343–352 (1988).



# Instructions for Use

## High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. **DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED.** Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS\* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

### Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).

Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).

Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).

Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

\* In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).

### Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.