

# Product Datasheet

## ERManI Antibody (3C2) NBP2-13167

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP2-13167**

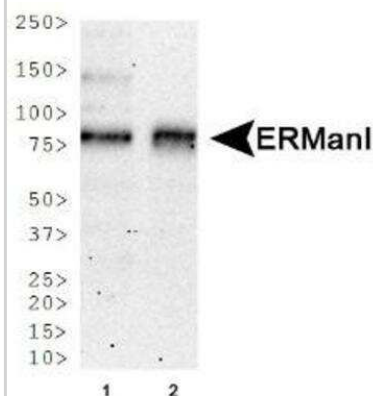
ERManI Antibody (3C2)

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	3C2
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	Tris-Glycine (pH 7.5) and 0.15M NaCl
Product Description	
Host	Mouse
Gene ID	11253
Gene Symbol	MAN1B1
Species	Human, Mouse (Negative)
Reactivity Notes	Does not react with mouse.
Immunogen	Full length human recombinant ERManI [Swiss-Prot# Q9UKM7]
Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot 1:500-1:1000, ELISA 1:1000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:10-1:100, Immunohistochemistry-Paraffin 1:200, Knockout Validated reported in scientific literature (PMID 26205822)
Application Notes	In ICC/IF, endoplasmic reticulum membrane staining was observed in HeLa cells. In Western Blot, a band is seen ~90 kDa representing ERManI. In IHC-P, staining was observed in the endoplasmic reticulum of human breast cancer tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

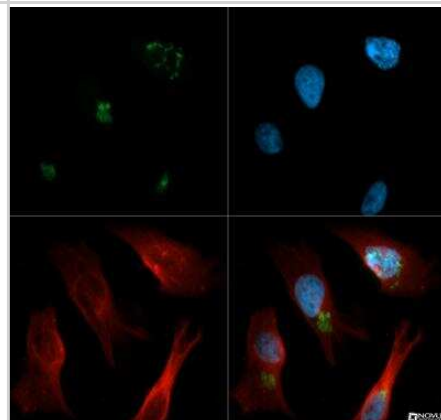


## Images

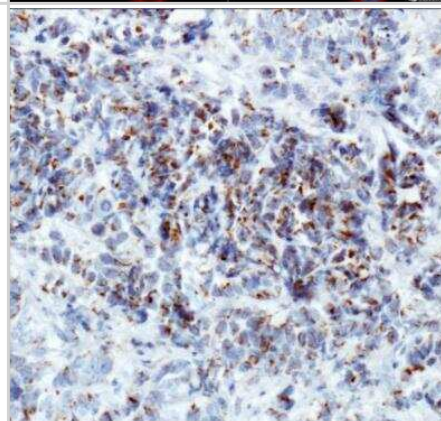
Western Blot: ERMAnI Antibody (3C2) [NBP2-13167] - Western blot analysis of ERMAnI expression in 1) NTERA-2 and 2) A-431 whole cell lysates.



Immunocytochemistry/Immunofluorescence: ERMAnI Antibody (3C2) [NBP2-13167] - ERMAnI antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



Immunohistochemistry: ERMAnI Antibody (3C2) [NBP2-13167] - IHC staining of ERMAnI in human breast cancer using DAB with hematoxylin counterstain.



## Publications

Frabutt DA, Wang B, Riaz S et al. Innate sensing of influenza A virus hemagglutinin glycoproteins by the host endoplasmic reticulum (ER) stress pathway triggers a potent antiviral response via ER-associated protein degradation. *J. Virol.* 2017-10-18 [PMID: 29046440] (Human)

Zhou T, Frabutt DA, Moremen KW, Zheng YH et al. ERMAnI is required for HIV-1 envelope glycoprotein degradation via endoplasmic reticulum-associated protein degradation pathway *J. Biol. Chem.* 2015-07-23 [PMID: 26205822] (WB, Human)

Rymen D, Peanne R, Millon MB et al. MAN1B1 Deficiency: An Unexpected CDG-II. *PLoS Genet* 2013-12-01 [PMID: 24348268] (WB, Human)

## Procedures

### Western blot Protocol Specific for ERManI Antibody (3C2) [NBP2-13167]

ERManI Antibody (3C2):

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
  2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
  3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
  4. Rinse the blot.
  5. Block the membrane using standard blocking buffer for at least 1 hour.
  6. Wash the membrane in wash buffer three times for 10 minutes each.
  7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
  8. Wash the membrane in wash buffer three times for 10 minutes each.
  9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
  10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
  11. Apply the detection reagent of choice in accordance with the manufacturers instructions.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

### Immunocytochemistry/Immunofluorescence protocol for ERManI Antibody (NBP2-13167)

ERManI Antibody (3C2):

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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**Immunohistochemistry-Paraffin Embedded Sections Protocol Specific for ERMα1 Antibody (3C2) [NBP2-13167]**

ERMα1 Antibody (3C2):

Immunohistochemistry-Paraffin Embedded Sections

**Antigen Unmasking:**

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

**Staining:**

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

[www.novusbio.com](http://www.novusbio.com)  
Technical Support: [nb-technical@bio-techne.com](mailto:nb-technical@bio-techne.com)  
Orders: [nb-customerservice@bio-techne.com](mailto:nb-customerservice@bio-techne.com)  
General: [novus@novusbio.com](mailto:novus@novusbio.com)

### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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