

## Product datasheet for **AM32179PU-N**

### Endothelium Mouse Monoclonal Antibody [Clone ID: EN4]

#### Product data:

Product Type: Primary Antibodies

Clone Name: EN4

Applications: IHC

Recommended Dilution: **Cell Cultures.**

**FACS/Flow Cytometry.**

**Immunohistochemistry on Frozen Sections.**

**Immunohistochemistry on Paraffin Sections** (1/20-1/100).

*Recommendation Use:* The antibody is useful for immunohistological staining of endothelia in Frozen sections. Lymphatic endothelium is stained (unlike the PAL-E antibody).

Kaposi's sarcoma and other vascular tumors of endothelial cell origin are also stained.

The antibody works well on acetone fixed or unfixed cryostat sections, and periodate/lysine/paraformaldehyde-fixed sections, in which membrane antigens and the structures of vessels and adjacent tissues are optimally preserved. See for further Instructions G and H.

Reactivity: Feline, Guinea Pig, Human

Host: Mouse

Isotype: IgG1

Clonality: Monoclonal

**Specificity:** This antibody stains all Human blood vessels including brain microvessels. Both large and small vessels are equally reactive. The EN4 antigen is preserved in endothelial cells in culture unlike the PAL-E antigen which is lost. It stains strongly murine fibroblasts transfected with the Human CD31 gene. On surface-iodinated Jurkat T cells it recognizes the 130 kD CD31 antigen.

Clone *EN4* also binds to Guinea-Pig and Cat endothelium, but **not** to Rabbit, Bovine, Sheep, Dog, Rat or Mice endothelial cells.

No other structures in the skin, heart, kidney, tonsils or spleen are stained with this antibody.

**Formulation:** PBS

State: Purified

State: Liquid (0.2 µm filtered) IgG fraction

Stabilizer: 0.1% BSA

Preservative: 0.02% Sodium Azide



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<b>Concentration:</b>	lot specific
<b>Conjugation:</b>	Unconjugated
<b>Storage:</b>	Store the antibody undiluted at 2-8°C.
<b>Stability:</b>	Shelf life: one year from despatch.
<b>Background:</b>	The <b>Endothelium</b> is located at the interface between the blood and the vessel wall. The cells are in close contact and form a slick layer that prevents blood cell interaction with the vessel wall as blood moves through the vessel lumen. The endothelium consists of simple squamous epithelium that lines the lumen of all blood vessels. It plays a critical role in the mechanics of blood flow, the regulation of coagulation, leukocyte adhesion, and vascular smooth muscle cell growth, and also serves as a barrier to the transvascular diffusion of liquids and solutes. For years the endothelium was thought of as an inert single layer of cells that passively allow the passage of water and other small molecules across the vessel wall. However, this dynamic tissue performs many other active functions, such as the secretion and modification of vasoactive substances and the contraction and relaxation of vascular smooth muscle.
<b>Synonyms:</b>	endothelial cells, endothelial marker
<b>Note:</b>	Protocol: <b><u>INSTRUCTIONS FOR USE-G</u></b>

#### ***INDIRECT IMMUNOPEROXIDASE STAINING ON FROZEN SECTIONS***

1. 4 to 6 micron thick sections should be used.
2. Sections are thawed, 1-2 hours at room temperature.
3. Tissue is fixed in acetone, 10 minutes.
4. Wash with PBS, 2 x 3 minutes.
5. Incubate with monoclonal antibody (diluted in PBS), 1-2 hours at room temperature.
6. Wash with PBS, 3 x 3 minutes.
7. Incubate with peroxidase labeled second antibody, 30-60 minutes at room temperature.
8. Wash with PBS, 3 x 3 minutes.
9. Stain with diaminobenzidin (DAB) solution 10 minutes at room temperature.
10. Wash with running tap water, 3 minutes.
11. Counterstain with Mayer's hematoxylin, 2 minutes.
12. Wash with running tap water, 5 minutes.
13. Dehydrate with increasing solution of ethanol; 50%, 70%, 96%, absolute, 3 minutes each.
14. Clear with xylol, 3 x 3 minutes.
15. Mount with mounting medium (e.g. malinol).

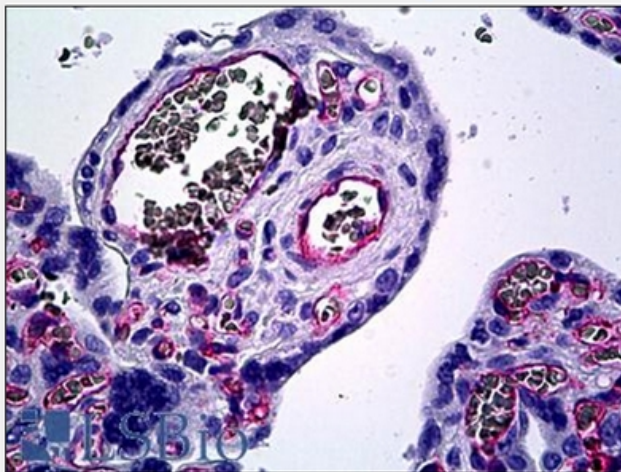
#### **INSTRUCTIONS FOR USE-H**

#### ***INDIRECT IMMUNOPEROXIDASE STAINING ON FORMALIN-FIXED AND PARAFFIN EMBEDDED TISSUES***

1. 4 micron thick sections should be used.
2. Dewax in xylol, 3 x 3 minutes.
3. Rehydrate in decreasing grades of ethanol: absolute, 96%, 70%, 50%, 3 minutes each.

4. Block endogenous peroxidase activity with freshly made 0.3% H<sub>2</sub>O<sub>2</sub> in methanol, 20 minutes.
5. Wash with PBS, 3 x 3 minutes.  
Only if trypsinization is required
- 5a. Incubate sections with 0.1% Trypsin in 0.1% CaCl<sub>2</sub> pH 7.6 for 10 minutes at room temperature.
- 5b. Wash with PBS, 3 x 3 minutes.
6. Cover the sections with 20% normal rabbit serum in PBS or normal human serum and incubate overnight in a humidity chamber at room temperature to reduce non specific background staining.
7. Decant 20% normal rabbit serum.
8. Incubate with monoclonal antibody (diluted in PBS), 1-2 hours at room temperature.
9. Wash with PBS, 3 x 3 minutes.
10. Incubate with peroxidase labeled second antibody, 30-60 minutes at room temperature.
11. Wash with PBS, 3 x 3 minutes.
12. Stain with diaminobensidin (DAB) solution, 10 minutes at room temperature. A stock solution of 0.5% DAB in 0.5M Tris/HCl (pH7.4) can be made and stored frozen in the dark. Before use a quantity needed for staining can be thawed and diluted 10x with water. The diluted DAB solution should be filtrated. Just before use H<sub>2</sub>O<sub>2</sub> must be added to a final concentration of 0.01%.
13. Wash with running tap water, 3 minutes.
14. Counterstain with Mayer's hematoxylin, 2 minutes.
15. Wash with running tap water, 2 minutes.
16. Dehydrate with increasing solutions of ethanol:50%, 70%, 96%, absolute, 3 minutes each.
17. Clear with xylol, 3 x 3 minutes.
18. Mount with mounting medium (e.g. malinol).

### Product images:



Human Placenta, Endothelium: Formalin-Fixed, Paraffin-Embedded (FFPE)