NEUROMICS

Endoglin/CD105 Data Sheet

Catalog Number:	MO15124	**Host:**	Rat IgG2a
Product Type:	Monoclonal Rat IgG2a Clone # 209701	**Species Reactivity:**	Mouse
Immunogen Sequence:	Mouse myeloma cell line NS0 derived recombinant mouse Endoglin Glu21Gly581 (predicted) Accession # NP_031958	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose

Applications:
Immunohistochemistry -8-25 µg/mL
Immunocytochemistry- 8-25 µg/mL
Western Blot-1 µg/mL
Flow Cytometry-.2.5 µg/10^6 cells-MS-1 mouse pancreatic islet endothelial cell line

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

Application Notes

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Immunohistochemistry:

Note: When staining cryostat sections stored in a freezer, thaw the slides at room temperature for 10-20 minutes.

1. Rehydrate the slides in wash buffer for 10 minutes. Drain the excess wash buffer.

Note: Excessive fixation may result in the masking of an epitope and strong non-specific background signal that can obscure specific labeling. If necessary, an antigen retrieval protocol can be performed at this time. However, many antigen retrieval techniques are too harsh for cryostat cut tissue sections.

2. Surround the tissue with a hydrophobic barrier using a barrier pen.
3. Block non-specific staining between the primary antibodies and the tissue, by incubating in blocking buffer (1% horse serum in PBS) for 30 minutes at room temperature.
4. Apply primary antibodies diluted in Incubation Buffer according to manufacturer's instructions. For fluorescent IHC staining of frozen tissue sections using R&D Systems antibodies, it is recommended to incubate overnight at 2-8 °C. This incubation regime allows for optimal specific binding of antibodies to tissue targets and reduces non-specific background staining. These variables may need to be optimized for your system.

Note: An isotype matched control can be performed when using monoclonal primary antibodies. In addition, a negative control using the incubation buffer with no primary antibody can be employed to identify non-specific staining.

5. Wash slides 3 times for fifteen minutes each in wash buffer.
6. Incubate with the NorthernLights secondary antibody diluted in Incubation Buffer according to the manufacturer's instructions. Recommended incubation with secondary antibody is for 30-60 minutes at room temperature. From this step forward samples should be protected from light.

Note: secondary antibodies and streptavidin conjugates are bright, resistant to photobleaching and are ideal for multi-color fluorescence microscopy.

Note: If a biotinylated antibody was used in step 5, apply the appropriate Streptavidin conjugate in step 7.

7. Wash slides 3 times for fifteen minutes each in Wash Buffer.
8. Add 300 µL of the diluted DAPI solution to each well, and incubate 2-5 minutes at room temperature. DAPI binds to DNA and is a convenient nuclear counterstain. It has an absorption maximum at 358 nm and fluoresces blue at an emission maximum of 461 nm.

Note: DAPI counterstain can obscure visualization of targets localized in cell nuclei.

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9. Rinse 1 time with PBS.
10. Mount with an anti-fade mounting media.
11. Visualize using a fluorescence microscope.

Image: Endoglin/CD105 was detected in immersion fixed frozen sections of mouse embryo (13.5 d.p.c.) using Rat Anti-Mouse Endoglin/CD105 Monoclonal Antibody at 10 µg/mL overnight at 4 °C. Tissue was stained using a 557-conjugated Anti-Rat IgG Secondary Antibody and counterstained with DAPI (blue). Specific staining was localized to endothelial cells of the developing hindlimb. *Image depicts staining at 10X magnification.*

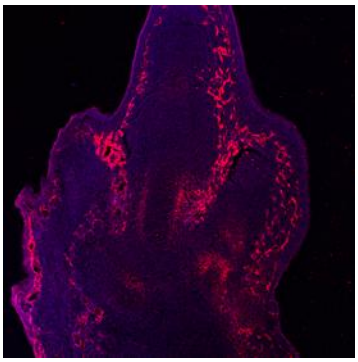
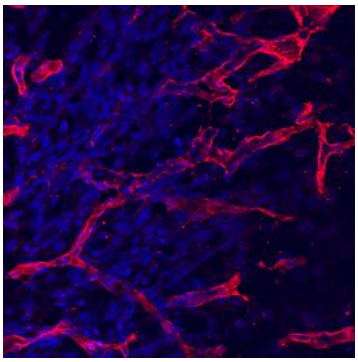


Image: Endoglin/CD105 was detected in immersion fixed frozen sections of mouse embryo (13.5 d.p.c.) using Rat Anti-Mouse Endoglin/CD105 Monoclonal Antibody at 10 µg/mL overnight at 4 °C. Tissue was stained using a 557-conjugated Anti-Rat IgG Secondary Antibody (red) and counterstained with DAPI (blue). Specific staining was localized to endothelial cells of the developing hindlimb. *Image depicts staining at 40X magnification.*



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