NEUROMICS

CD34/HPCA1 Data Sheet

Catalog Number:	MO20026	**Host:**	Mouse
Ig Class:	IgG_1, Clone: QBEnd/10	**Species Reactivity:**	Human
Immunogen Sequence:	Detergent solubilized vesicular suspension prepared from a perfusate of human term placenta.	**Format:**	Liquid- tissue culture supernatant containing 15mM sodium azide. Concentration : Approximately .05 mg/ml. Specific concentration on vial label

Applications: Immunohistochemistry on paraffin sections. Suggested dilution: 1:50 for 60 minutes at 25oC. Trypsin digestion is recommended.

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

Total Protein Concentration 1.0 - 8.0 g/L. Refer to vial label for specific concentration.

This antibody is specific for the Human CD34 antigen.

Description/Data:
CD34 is a type I transmembrane glycophosphoprotein expressed by hematopoietic stem/progenitor cells (HSCs). CD34 may also stimulates proportions of adult human HSCs to differentiate into full-fledged neurons. This may open new possibilities for a high-yield production of neurons from bone marrow.

In tumors, CD34 is found in alveolar soft part sarcoma, preB-ALL (positive in 75%), AML (40%), AML-M7 (most), dermatofibrosarcoma protuberans, gastrointestinal stromal tumors, giant cell fibroblastoma, granulocytic sarcoma, Kaposi's sarcoma, liposarcoma, malignant fibrous histiocytoma, malignant peripheral nerve sheath tumors, mengingeal hemangiopericytomas, meningiomas, neurofibromas, schwannomas, and papillary thyroid carcinoma.

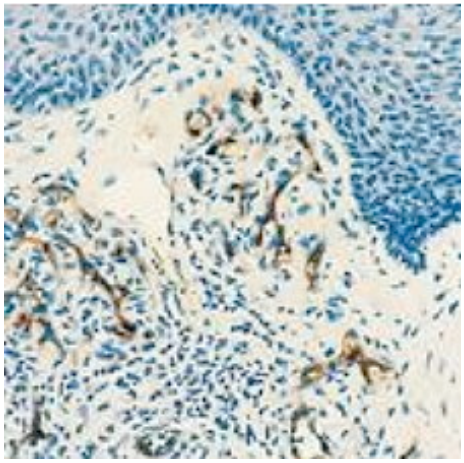

Image: CD34 staining of human tonsil tissue. Note intense staining of neoplastic endothelial cells and absence of staining of stromal cells. Paraffin section.

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Immunohistochemistry:

A. Reagents required but not supplied

1.Standard solvents used in immunohistochemistry.
2.Hydrogen peroxide.
3.50mM Tris-buffered saline (TBS) pH7.6.
4.Antigen retrieval solution(s) - see **Recommendations on Use**.
5.Antibody diluent - optimally diluted normal serum.
6.Normal sera from the species in which the secondary antibody is raised.
7.Secondary biotinylated antibody.
8.Avidin/Biotin Complex-Horseradish peroxidase (ABC-HRP) - prepare as recommended by manufacturer.
9.3,3' Diaminobenzidine tetrahydrochloride (DAB) - prepare as recommended by manufacturer.
10.Hematoxylin counterstain - prepare as recommended by manufacturer.
11.Mounting medium - use as recommended by manufacturer.

B. Equipment required but not supplied

1.Incubator set to 25°C.
2.Water bath set to 37°C.
3.General immunohistochemistry laboratory equipment.

C. Antigen retrieval solutions

Trypsin enzyme solution
Preheat the following to 37°C using a water bath:
0.2L distilled water.
0.2L TBS.
Dissolve 0.2g trypsin 250 (DIFCO product code 0152-13) and 0.2g calcium chloride in 0.2L TBS.
When the trypsin enzyme solution is at 37°C, pH to 7.8 with 0.1M sodium hydroxide.

D. Methodology

Customers should determine optimal dilutions for antibodies. Unless indicated, all steps are performed at room temperature (25°C).
1.Cut and mount sections on slides coated with a suitable tissue adhesive.
2.De-paraffinize sections in xylene or xylene substitutes.
3.Re-hydrate through graded alcohols.
4.Neutralize endogenous peroxidase using 0.5%v/v hydrogen peroxide/methanol for 10 minutes.
5.Wash slides in running tap water.
Pretreat the sections as follows:
6.Place the slides in the preheated (37°C) distilled water to warm the sections for a minimum of 5 minutes.
7.Incubate in trypsin enzyme solution at 37°C for 30 minutes8.Wash sections in TBS for 1 x 5 minutes with gentle rocking.
9.Cover sections with diluted normal serum for 10 minutes.
10.Incubate sections with optimally diluted primary antibody
11.Wash in TBS buffer for 2 x 5 minutes with gentle rocking.
12.Incubate sections in appropriate biotinylated secondary antibody.
13.Wash in TBS buffer for 2 x 5 minutes with gentle rocking.
14.Incubate slides in ABC-HRP.
15.Wash in TBS buffer for 2 x 5 minutes with gentle rocking.
16.Incubate slides in DAB.
17.Rinse slides in water.
18.Counterstain with hematoxylin.
19.Dehydrate, clear and mount sections.

Positive Tissue Control

Used to indicate correctly prepared tissues and proper staining techniques. One positive tissue control should be included for each set of test conditions in each staining run. A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation. Recommended positive control tissue is tonsil (vascular elements). If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

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Negative Tissue Control
Should be examined after the positive tissue control to verify the specificity of the labelling of the target antigen by the primary antibody. Recommended negative control tissue is tonsil (non-vascular elements). Alternatively, the variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user. Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain non-specifically.3 False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by endogenous enzymes such as pseudoperoxidase (erythrocytes), endogenous peroxidase (cytochrome C), or endogenous biotin (eg. liver, breast, brain, kidney) depending on the type of immunostain used. To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate chromogen or enzyme complexes (avidin-biotin streptavidin, labelled polymer) and substrate-chromogen, respectively. If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid.

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