

# CD34

Concentrated and Prediluted Monoclonal Antibody  
901-084-052423

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 084 A, B, C	0.1, 0.5, 1.0 mL	1:100	Da Vinci Green
Predilute	PM 084 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IP 084 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 084 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 084 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 084 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 084 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

CD34 [QBEnd/10] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CD34 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

## Summary and Explanation:

CD34 recognizes a single chain transmembrane glycoprotein of 110 kDa. This antigen is selectively expressed in human lymphoid and myeloid hematopoietic progenitor cells. The antibody to CD34 also reacts with vascular endothelial cells in normal tissues, and in benign and malignant proliferations. The utility of CD34 has value in the studies of benign and malignant vascular tumors as well as characterization of acute leukemia in bone marrow. CD34 has been used to measure angiogenesis in many types of tumors, which reportedly predicts tumor recurrence. It is also used to differentiate dermatofibrosarcoma protuberans from fibrous histiocytoma.

## Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** QBEnd/10

**Isotype:** IgG1

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** CD34

**Cellular Localization:** Cell surface and cytoplasmic

**Positive Tissue Control:** Tonsil, skin or angiosarcoma

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

## Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Warp Red.

## Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

## intelliPATH FLX Automated Slide Stainer:

IP084 is intended for use on the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

## Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI084 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** CD34

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR1, high pH; 101°C

**Reagent Name, Time, Temp.:** CD34, 30 min., 25°C

## Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI084 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** CD34

**Protocol Template (Description):** Mouse HRP Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** CD34, 1 hour, 25°C

## Protocol Recommendations (Ventana BenchMark ULTRA):

AVI084 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 32 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

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**Primary Antibody:** 16 minutes, 36°C

### **Protocol Recommendations (Q Series – For Leica BOND-III):**

ALI084 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

#### **- DAB Chromogen Staining Option:**

**Protocol Name:** IHC Protocol F

**Detection:** Bond Polymer Refine

**HIER:** 20 min with ER1

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary:** 8 min

**Polymer:** 8 min

**Mixed DAB Refine:** 10 min

**Hematoxylin:** 5 min

#### **- Red Chromogen Staining Option:**

**Protocol Name:** IHC Protocol J

**Detection:** Bond Polymer Refine Red

**HIER:** 10 min with ER2

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary AP:** 20 min

**Polymer AP:** 30 min

**Mixed Red Refine:** 10 min + 5 min

**Hematoxylin:** 5 min

### **Limitations:**

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

### **Quality Control:**

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA ([www.clsi.org](http://www.clsi.org)). 2011

### **Precautions:**

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (8)

### **Precautions Cont'd:**

3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

### **Troubleshooting:**

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

### **References:**

1. Rimsza LM, *et al.* The presence of CD34+ cell clusters predicts impending relapse in children with acute lymphoblastic leukemia receiving maintenance chemotherapy. *Am J Clin Pathol.* 1998 Sep;110(3):313-20.
2. Bettencourt MC, *et al.* CD34 immunohistochemical assessment of angiogenesis as a prognostic marker for prostate cancer recurrence after radical prostatectomy. *J Urol.* 1998 Aug;160(2):459-65.
3. Sauter B, *et al.* Immunoelectron microscopic characterization of human dermal lymphatic microvascular endothelial cells. Differential expression of CD31, CD34, and type IV collagen with lymphatic endothelial cells vs blood capillary endothelial cells in normal human skin, lymphangioma, and hemangioma in situ. *J Histochem Cytochem.* 1998 Feb;46(2):165-76.
4. Diaz-Casajo C, *et al.* Dermatofibrosarcoma protuberans with fibrosarcomatous areas: a clinico-pathologic and immunohistochemic study in four cases. *Am J Dermatopathol.* 1997 Dec;19(6):562-7.
5. Yamane H, *et al.* Small cell lung cancer can express CD34 antigen. *Anticancer Res.* 1997 Sep;17(5A):3627-32.
6. Raspadori D, *et al.* Incidence and prognostic relevance of CD34 expression in acute myeloblastic leukemia: analysis of 14 cases. *Leuk Res.* 1997 Jul;21(7):603-7.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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