CONFIRM anti-CD34 (QBEnd/10) Primary Antibody

REF 790-2927

05278210001

IVD Σ 50

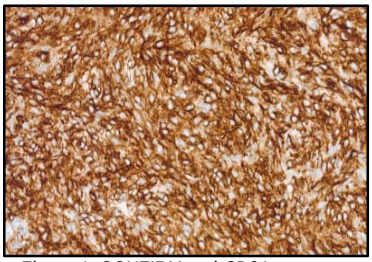

Figure 1. CONFIRM anti-CD34 (QBEnd/10) Primary Antibody staining of GIST.

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-CD34 (QBEnd/10) Primary Antibody is a mouse monoclonal antibody (IgG1) directed against human CD34. This antibody is intended for use to qualitatively identify CD34 by light microscopy in sections of formalin fixed, paraffin embedded tissue stained on a Ventana automated slide stainer.

The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

This antibody is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

CD34 was identified in 1984 as a marker of early hematopoietic cells, including hematopoietic stem cells, common myeloid progenitor (CMP) cells, colony-forming unit-megakaryocyte (CFU-MK) cells, granulocyte-monocyte progenitor (GMP) cells, monoblasts, myeloblasts, and promegakaryoblasts.[1,2] Studies implicate CD34 as an anti-adhesion molecule, which has downstream effects on cell migration and tissue permeability.[3,4,5] CD34 is also known to interact with adhesion molecule L-selectin as well as CRK-like proto-oncogene (CRKL), an adaptor protein that can activate the RAS and JUN kinase oncogenic signaling pathways.[6,7,8]

Expression of CD34 in hematopoietic stem and progenitor cells makes this marker useful to determine tissue lineage when used in combination with other markers, however CD34 is not restricted to the hematopoietic lineage.[3,9] Cell types outside of the hematopoietic lineage that express CD34 include vascular endothelial tissue, fibroblasts, and muscle progenitor cells.[4,9,10,11,12] Detection of CD34 is commonly used as an aid in diagnosis soft tissue tumors, including those of vascular endothelial origin and dermatofibrosarcoma protuberans (DFSP), which is derived from fibroblasts.[9,11,12] Of note, CD34 expression is also seen in a subset of interstitial cells of Cajal, mesenchymal cells that act as pacemaker cells in the gastrointestinal tract and mediate communication between smooth muscle and the autonomic nervous system.[9,12,13] The expression of CD34 is commonly seen in gastrointestinal stromal tumors (GISTs), which likely originate from interstitial cells of Cajal.[12,13]

Clinical applications for the detection of CD34 by Immunohistochemistry (IHC) with the CONFIRM anti-CD34 (QBEnd/10) Primary Antibody include: aid in the identification of blasts in normal and neoplastic tissue; aid in the diagnosis of soft tissue tumors of vascular endothelial origin; aid in the diagnosis of dermatofibrosarcoma protuberans (DFSP); and aid in the diagnosis of gastrointestinal stromal tumor (GIST). This antibody may be used as part of a panel of IHC studies. The staining pattern is membranous.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD34 (QBEnd/10) Primary Antibody may be used as the primary antibody for IHC staining of formalin-fixed, paraffin-embedded (FFPE) tissue sections. CONFIRM anti-CD34 (QBEnd/10) Primary Antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-CD34 (QBEnd/10) Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD34 (QBEnd/10) Primary Antibody contains approximately 4 µg of a mouse monoclonal antibody directed against CD34 present in tissue.

The antibody is diluted in a buffer containing carrier protein and preservative.

Specific antibody concentration is approximately 0.8 µg/mL. CONFIRM anti-CD34 (QBEnd/10) Primary Antibody is a mouse IgG. There is no known non-specific antibody reactivity observed in this product.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[14] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[15,16]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to the tables below for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-2927.

Table 2. Recommended staining protocol for CONFIRM anti-CD34 (QBEnd/10) Primary Antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1 8 minutes	CC1 8 minutes	ULTRA CC1 8 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	8 minutes, 37°C	8 minutes, 37°C	8 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for CONFIRM anti-CD34 (QBEnd/10) Primary Antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1 Short	CC1 Short	ULTRA CC1 Short
Antibody (Primary)	12 minutes, 37°C	12 minutes, 37°C	12 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[17]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-CD34 (QBEnd/10) Primary Antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. A tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, and not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

An example of a positive control for CONFIRM anti-CD34 (QBEnd/10) Primary Antibody is spleen. The positive staining tissue components (membrane and cytoplasmic staining of vascular endothelial cells) are used to confirm that the antibody was applied and the instrument functioned properly.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-CD34 (QBEnd/10) Primary Antibody is membranous.

This antibody may demonstrate staining of endothelial cells, stroma and blasts in normal and neoplastic tissues.

SPECIFIC LIMITATIONS

OptiView detection system is generally more sensitive than *ultra*View detection system.

The user must validate the results obtained with this reagent and detection systems.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Normal endothelial cells and/or stroma are expected to exhibit positive membranous staining for CD34 in the majority of normal and neoplastic tissues while all other cellular elements are expected to be negative. CD34 can also identify normal endothelial cells, stroma and/or blasts in bone marrow.

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-CD34 (QBEnd/10) Primary Antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum [a]	2/3	Bone marrow [b]	3/3
Cerebellum [a]	3/3	Lung [a]	3/3
Adrenal gland [a]	3/3	Heart [a]	8/8
Ovary [a]	3/3	Esophagus [a]	3/3
Pancreas [a]	3/3	Stomach [a]	4/4
Lymph node [a]	4/4	Small intestine [a]	9/9
Parathyroid gland	0/3	Colon [a]	3/3
Pituitary gland [a]	3/3	Liver [a]	3/3
Testis [a]	3/3	Salivary gland [a]	3/3
Thyroid [a]	3/3	Kidney [a]	3/3
Breast [a]	3/3	Prostate [a]	3/3
Spleen [a]	5/5	Cervix [a]	3/3
Tonsil [a]	3/3	Skin [a]	3/3
Endometrium [a]	3/3	Bladder	0/3
Skeletal muscle [a]	3/3	Vessels, arteries and veins [a]	3/3
Nerve [a]	2/3	Placenta [a]	2/2
Mesothelium [a]	3/3	Umbilical cord [a]	2/2
Thymus [a]	3/3		

[a] Positive cases exhibited targeted membranous staining in the endothelial cells and/or stroma while all other cellular elements were negative.

[b] Positive cases exhibited staining in the endothelial cells and blasts while all other cellular elements were negative.

Table 5. Sensitivity/Specificity of CONFIRM anti-CD34 (QBEnd/10) Primary Antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/2
Meningioma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Angioleiomyoma (Nasal cavity)	1/1
Hemangioma (Nasal cavity)	1/1
Endometrioid carcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
B-cell Lymphoma; NOS (Spleen)	0/1
Hemangioma (Spleen)	1/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach)	0/1
Gastrointestinal stromal tumor (GIST) (Stomach)	21/22
Adenocarcinoma (Small intestine)	0/31
Malignant Mixed Mesenchymal Neoplasm (Small intestine)	0/1
GIST (Small intestine)	33/43
Carcinoid (Small intestine)	0/2
Neuroendocrine carcinoma (Small intestine)	0/1
Sarcomatoid carcinoma (Small intestine)	0/1
Metastatic gastric carcinoma (Small intestine)	0/1
B-cell lymphoma, NOS (Small intestine)	0/4
Diffuse Large B-cell Lymphoma (Small intestine)	0/6

Pathology	# positive / total cases
Non-Hodgkin lymphoma, NOS (Small intestine)	0/3
T-cell lymphoma, NOS (Small intestine)	0/1
Leiomyosarcoma (Small intestine)	0/2
Adenocarcinoma (Colon)	0/1
Malignant Mixed Mesenchymal Neoplasm (Colon)	1/1
Adenocarcinoma (Rectum)	0/1
Malignant Mixed Mesenchymal Neoplasm (Rectum)	1/1
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Hemangioma (Liver)	2/2
Clear cell carcinoma (Kidney)	0/1
Angioleiomyoma (Kidney)	1/1
Hemangioma (Kidney)	1/1
Myxoma (Heart)	2/2
Mesothelial sarcoma (Pericardium)	2/2
Paraganglioma (Carotid body)	1/1
Hemangiopericytosarcoma (Abdomen wall)	1/1
Adenocarcinoma (Prostate)	0/2
Leiomyosarcoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Hemangioma (Skin)	2/2
Neurofibroma (Nerve)	1/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	0/1
Pleomorphic rhabdomyosarcoma (Peritoneum)	0/1
B-cell lymphoma, NOS (Lymph node)	0/2
Hodgkin Lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Leiomyosarcoma (Smooth muscle)	0/1
Leiomyoma	3/9
Leukemia	2/2
Dermatofibrosarcoma protuberans	8/8

Precision

Precision studies for CONFIRM anti-CD34 (QBEnd/10) Primary Antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.
  All studies met their acceptance criteria.
- Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision.
  All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of CONFIRM anti-CD34 (QBEnd/10) Primary Antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

1. Civin CI. A Hematopoietic Progenitor Cell Surface Antigen Defined By Monoclonal Antibody Raised Against KG1a Cells. J Immunol. 1984;133:157-165.
2. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Vol 2. Revised 4th ed2017.
3. Nielsen JS, McNagny KM. Novel functions of the CD34 family. J Cell Sci. 2008;121(Pt 22):3683-3692.
4. Nielsen JS, McNagny KM. CD34 is a key regulator of hematopoietic stem cell trafficking to bone marrow and mast cell progenitor trafficking in the periphery. Microcirculation. 2009;16(6):487-496.
5. Ohnishi H, Sasaki H, Nakamura Y, et al. Regulation of cell shape and adhesion by CD34. Cell Adh Migr. 2013;7(5):426-433.
6. Felschow DM, McVeigh ML, Hoehn GT, Civin CI, Fackler MJ. The adapter protein CrkL associates with CD34. Blood. 2001;97(12):3768-3775.
7. Baumhueter S, Singer MS, Henzel W, et al. Binding of L-Selectin to the Vascular Sialomucin Cd34. Science. 1993;262(5132):436-438.
8. Blanchet MR, Maltby S, Haddon DJ, Merkens H, Zbytnuik L, McNagny KM. CD34 facilitates the development of allergic asthma. Blood. 2007;110(6):2005-2012.
9. Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cells. 2014;32(6):1380-1389.
10. Fina L, Molgaard HV, Robertson D, et al. Expression of the CD34 gene in vascular endothelial cells. Blood. 1990;75(12):2417-2426.
11. Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. Blood. 1996;87(1):1-13.
12. Goldblum JR, Folpe AL, Weiss SW. Enzinger and Weiss's Soft Tissue Tumors, 7th Edition. 7th ed. Philadelphia, PA: Elsevier; 2020.
13. Miettinen M, Lasota J. Gastrointestinal stromal tumors (GISTs): definition, occurrence, pathology, differential diagnosis and molecular genetics. Pol J Pathol. 2003;54(1):3-24.
14. Sheehan DC, Hrapchak BB. Theory and practice of histotechnology, 2nd Edition. The C.V. Mosby Company, St. Louis, 1980.
15. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
16. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work
17. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
F	Updates to Warnings and Precautions section. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, AZ 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany
+800 5505 6606

CE 0123