CD68 (SP251v18) Rabbit Monoclonal Antibody

REF 790-7248

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IVD Σ 50

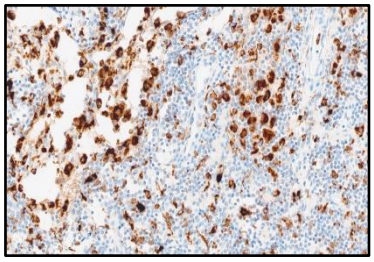

Figure 1. CD68 (SP251v18) antibody staining of macrophages in the lymph node.

INTENDED USE

CD68 (SP251v18) Rabbit Monoclonal Antibody is intended for laboratory use in the qualitative immunohistochemical detection of CD68 by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

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

SUMMARY AND EXPLANATION

Cluster of differentiation 68 (CD68) is a member of a broad family of proteins known as lysosomal/endosomal-associated membrane glycoproteins (LAMPs).[1,2] The antigen is a 110 kD integral membrane glycoprotein with one LAMP domain, a transmembrane domain, and a short cytoplasmic tail that holds motifs important for localizing to lysosomes.[1,2] CD68 molecule is also known as scavenger receptor class D member 1, CD68 antigen, and macrophage antigen CD68.[1,2] The function of CD68 is unknown, however evidence suggests that it inhibits antigen uptake, loading, or trafficking of major histocompatibility complex class II (MHC-II).[1-4]

CD68 is highly expressed by monocytes and tissue macrophages, such as histiocytes, osteoclasts, and Kupffer cells, and is expressed to a lesser extent in other cells of the myeloid lineage, such as dendritic cells.[1] Though initially identified in macrophages, CD68 expression has since been tied to the lysosomal and endosomal content of cells.[5] Because of this, CD68 expression is not restricted to the myeloid lineage, and expression is seen in other hematopoietic and non-hematopoietic cell types, such as fibroblasts and some T lymphocytes.[6-8] Clinically, the high lysosomal content of macrophages and monocytes makes CD68 a useful marker to identify areas of inflammation and immune infiltration of tumors.[1, 9-11]

The detection of CD68 by immunohistochemistry (IHC) with the CD68 (SP251v18) Rabbit Monoclonal Antibody (CD68 (SP251v18)) may be used as an aid in the identification of macrophages in normal or neoplastic tissue.

PRINCIPLE OF THE PROCEDURE

CD68 (SP251v18) binds to CD68 in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using the OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or the *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheets for further information.

MATERIAL PROVIDED

CD68 (SP251v18) contains sufficient reagent for 50 tests.

One 5 mL dispenser of CD68 (SP251v18) contains approximately 6 µg of a rabbit monoclonal antibody.

The antibody is diluted in a phosphate buffer containing carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 1.2 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CD68 (SP251v18) is a recombinant rabbit monoclonal antibody produced as a purified cell culture supernatant

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. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
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. General purpose laboratory equipment
15. 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.[12] 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.

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.[13,14]
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.
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	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, a 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 790-7248.

Table 2. Recommended staining protocol for CD68 (SP251v18) with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 32 minutes	ULTRA Cell Conditioning 1 32 minutes, 100°C
Pre Primary Peroxidase Inhibitor	Selected	
Antibody (Primary)	32 minutes, 37 °C	16 minutes, 36 °C
OptiView HQ Linker	8 minutes	
OptiView HRP Multimer	8 minutes	
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 CD68 (SP251v18) with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 30 Minutes (Mild)	ULTRA Cell Conditioning 1 36 minutes (Mild), 95°C
Antibody (Primary)	16 minutes, 37 °C	16 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."[15]

NEGATIVE REAGENT CONTROL

In addition to staining with CD68 (SP251v18), a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

A positive tissue control must be included with each staining run. 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. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining 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 performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An example of a positive control tissue for this antibody is normal tonsil. The positive staining tissue components (macrophages) are used to confirm that the antibody was applied and the instrument functioned properly.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CD68 (SP251v18) is membranous and / or cytoplasmic.

SPECIFIC LIMITATIONS

The OptiView DAB IHC Detection Kit is generally more sensitive than the *ultra*View Universal DAB Detection Kit. 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

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

Tissue in which resident macrophages are present, such as: tonsil, spleen, bone marrow (monocytes/myeloid precursors), liver (Kupffer cells), brain (microglial cells), and skin (histiocytes) show positive staining, therefore the tissue specimens are deemed positive. In these tissues, only the resident macrophages are staining, not the tissue parenchyma.

The remainder of the tissues have various degrees of infiltrating macrophages, which show positive staining, however the tissue specimens are deemed negative since the macrophages do not reside permanently in the tissue.

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Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CD68 (SP251v18) was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	4/4	Esophagus	0/4
Cerebellum	0/4	Stomach	0/4
Adrenal gland [a]	0/4	Small intestine	0/4
Ovary	0/4	Colon	0/4
Pancreas	0/4	Rectum	0/1
Lymph node [b]	4/4	Liver	4/4
Parathyroid gland	0/3	Salivary gland	0/4
Pituitary gland	0/3	Kidney [c]	0/8
Testis	0/4	Prostate [a]	0/4
Thyroid	0/4	Bladder	0/4
Breast	0/4	Endometrium	0/4
Spleen	3/3	Cervix	0/4
Tonsil	3/3	Skeletal muscle	0/3
Thymus	0/3	Skin	4/4
Bone marrow	3/3	Nerve	0/3
Lung	0/7	Mesothelium	0/3
Heart	0/3		

[a] Tissue evaluated includes normal and hyperplasia.

[b] Tissue evaluated includes normal and reactive.

[c] Tissue evaluated includes normal and inflammatory.

Table 5. Sensitivity/Specificity of CD68 (SP251v18) was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Malignant meningioma (Cerebellum)	0/1
Meningioma, fibroblastic (Brain)	1/2
Astrocytoma (Brain)	0/1
Squamous cell carcinoma (Head and neck)	0/4
Nasopharyngeal carcinoma (Nasopharynx)	0/1
Adenoma (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	0/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Endometrioid adenocarcinoma (Ovary)	0/1
Metastatic colon signet ring cell carcinoma (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Metastatic breast ductal carcinoma (Lymph node)	0/1
Metastatic esophagus squamous cell carcinoma (Lymph Node)	0/1
Seminoma (Testis)	0/2
Adenoma (Thyroid)	0/3
Follicular carcinoma (Thyroid)	0/1
Follicular adenocarcinoma (Thyroid)	0/1
Fibroadenoma (Breast)	0/2
Invasive ductal carcinoma (Breast)	0/3
Squamous cell carcinoma (Lung)	0/2
Adenocarcinoma (Lung)	2/61
Small cell carcinoma (Lung)	0/1
Metastatic cancer (Lung)	0/1
Adenosquamous carcinoma (Lung)	0/1
Adenocarcinoma in situ (Lung)	0/9
Papillary carcinoma (Lung)	0/3
Mucinous adenocarcinoma (Lung)	0/4
Adenocarcinoma (Stomach)	1/3
Adenoma (Small intestine)	0/1
Adenocarcinoma (Small intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/3
Adenocarcinoma (Rectum)	0/3
Hepatocellular carcinoma (Liver)	0/4
Metastatic colon adenocarcinoma (Liver)	0/1
Pleomorphic adenoma (Salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	0/1
Clear cell carcinoma (Kidney)	18/55
Oncocytoma (Kidney)	0/1
Papillary cell carcinoma (Kidney)	2/7
Chromophobe renal cell carcinoma (Kidney)	0/3
Medullary carcinoma (Kidney)	0/1
Granular cell carcinoma (Kidney)	3/9
Urothelial Carcinoma (Kidney)	0/12
Carcinosarcoma (Kidney)	0/1
Adenocarcinoma (Kidney)	0/1
Squamous cell carcinoma (Kidney)	0/4
Undifferentiated carcinoma (Kidney)	1/1
Nephroblastoma (Kidney)	0/3
Adenocarcinoma (Prostate)	0/2
Urothelial carcinoma (Bladder)	0/2
Adenocarcinoma (Endometrium)	0/2
Squamous cell carcinoma (Cervix)	0/2
Squamous cell carcinoma (Skin)	0/1
Melanoma	0/1
Hodgkin lymphoma	0/1
B-cell lymphoma, NOS	0/2

Pathology	# positive / total cases
Diffuse large B-cell lymphoma	0/1
Osteosarcoma (Bone)	0/5
Chondrosarcoma (Bone)	0/4
Adenocarcinoma (Bone)	0/1
Giant cell tumor (Bone)	10/10
Ameloblastoma (Bone)	0/2
Metastatic adenocarcinoma (Bone)	0/1
Metastatic carcinoma (Bone)	0/4
Rhabdomyosarcoma (Peritoneum)	0/1
Spindle cell sarcoma (Peritoneum)	0/1
Carcinosarcoma (Peritoneum)	0/1
Lipoma (Soft tissue)	0/1
Liposarcoma (Soft tissue)	0/16
Fibroma (Soft tissue)	0/2
Fibrosarcoma (Soft tissue)	0/20
Dermatofibrosarcoma protuberans (Soft tissue)	0/3
Embryonal rhabdomyosarcoma (Soft tissue)	0/3
Alveolar rhabdomyosarcoma (Soft tissue)	1/3
Polymorphic rhabdomyosarcoma (Soft tissue)	2/3
Leiomyosarcoma (Soft tissue)	1/9
Synovial sarcoma (Soft tissue)	0/3
Epithelioid sarcoma (Soft tissue)	0/2
Clear cell sarcoma (Soft tissue)	0/1
Malignant fibrous histiocytoma	3/4

Precision

Precision studies for CD68 (SP251v18) 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 ULTRA / BenchMark ULTRA PLUS instrument.
- Between platform precision between the BenchMark GX, BenchMark ULTRA / BenchMark ULTRA PLUS 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 instrument intermediate precision. All studies met their acceptance criteria.

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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
B	Update to Warnings and Precautions section.

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