CONFIRM anti-CD68 (KP-1) Primary Antibody

REF 790-2931

05278252001

IVD Σ 50

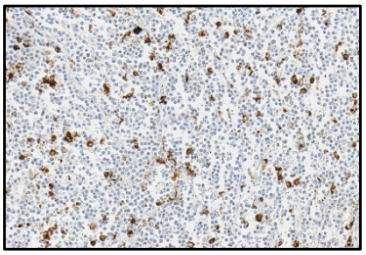

Figure 1. CONFIRM anti-CD68 (KP-1) antibody staining of macrophages in lymph node.

INTENDED USE

CONFIRM anti-CD68 (KP-1) Primary 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 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]

Although predominantly expressed in endosomal and lysosomal compartments of the cell, a smaller fraction of CD68 is located on the cell surface.[1,5,6,7] 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.[8] 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.[7,9,10] Clinically, the high lysosomal content of macrophages and monocytes makes CD68 a useful marker to identify areas of inflammation and immune infiltration of tumors.[1,6,11,12]

The clinical application for the detection of CD68 by immunohistochemistry with the CONFIRM anti-CD68 (KP-1) Primary Antibody (CONFIRM anti-CD68 (KP-1) antibody) is as an aid in the identification of macrophages in normal or neoplastic tissue.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD68 (KP-1) antibody is a mouse monoclonal antibody which 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 *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheets for further information.

MATERIAL PROVIDED

CONFIRM anti-CD68 (KP-1) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD68 (KP-1) antibody contains approximately 2 µg of a mouse monoclonal antibody

The antibody is diluted in phosphate buffered saline, with a carrier protein and a preservative.

Specific antibody concentration is approximately 0.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-CD68 (KP-1) antibody is a mouse monoclonal antibody produced as 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. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Prediluted) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Prediluted) (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. Mounting medium
15. Cover glass
16. General purpose laboratory equipment
17. 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.[13] 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. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. 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.
6. 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.
7. 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.[14,15]

8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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 dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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 dust/ fume/ gas/ mist/ vapours/ spray.
	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, 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 790-2931.

Table 2. Recommended staining protocol for CONFIRM anti-CD68 (KP-1) 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, 64 minutes	CC1, 64 minutes	ULTRA CC1 64 minutes, 100 °C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	4 minutes, 37 °C	4 minutes, 37 °C	4 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

Table 3. Recommended staining protocol for CONFIRM anti-CD68 (KP-1) 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, Standard	CC1, Standard	CC1, Standard
Antibody (Primary)	16 minutes, 37 °C	16 minutes, 37 °C	20 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."[16]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-CD68 (KP-1) 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. 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 with CONFIRM anti-CD68 (KP-1) antibody is 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 CONFIRM anti-CD68 (KP-1) antibody is membranous or cytoplasmic.

SPECIFIC LIMITATIONS

OptiView detection system is generally more sensitive than *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

Infiltrating macrophages, mast cells, Kupffer cells (liver tissue), microglial cells (brain), and monocytes are expected to stain positive in all tissues even when all other cellular components in the tissue are expected to be negative. All infiltrating macrophages that were present in tissues stained positive.

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-CD68 (KP-1) antibody was determined by testing FFPE normal tissues.

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

[a] Tissues evaluated includes normal and chronic inflammation.

Table 5. Sensitivity/Specificity of CONFIRM anti-CD68 (KP-1) 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
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
Invasive ductal carcinoma (Breast)	0/3
Diffuse large B-cell lymphoma (DLBCL) (Spleen)	0/1
Hemangioma (Spleen)	0/1
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)	0/3
Adenocarcinoma (Small intestine)	0/1
Mixed Malignant Mesenchymal Neoplasm (Small intestine)	0/1
GIST (Small intestine)	0/3
Adenocarcinoma (Colon)	0/1
Mixed Malignant Mesenchymal Neoplasm (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Mixed Malignant Mesenchymal Neoplasm (Rectum)	0/1
Melanoma (Rectum)	0/1
Hemangioma (Liver)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Adenocarcinoma (Kidney)	0/1
Chromophobe renal cell carcinoma (Kidney)	0/3
Medullary carcinoma (Kidney)	1/1
Nephroblastoma (Kidney)	0/2
Renal clear cell carcinoma (Kidney)	10/53
Granular cell carcinoma (Kidney)	2/9
Oncocytoma (Kidney)	0/1
Papillary cell carcinoma (Kidney)	2/7
Squamous cell carcinoma (Kidney)	0/3
Transitional cell carcinoma (Kidney)	3/12
Undifferentiated carcinoma (Kidney)	0/1
Wilms' tumor (Kidney)	0/1
DLBCL (Kidney)	0/2
Adenocarcinoma (Prostate)	0/2

Pathology	# positive / total cases
Leiomyoma (Uterus)	0/3
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Leiomyosarcoma	1/17
Squamous cell carcinoma (Cervix)	0/2
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Nerve)	0/1
Neuroblastoma (Retroperitoneum)	0/1
DLBCL (Lymph node)	1/2
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	1/1
Urothelial carcinoma (Bladder)	0/1
Osteosarcoma (Bone)	2/6
Giant cell tumor (Bone)	11/11
Ameloblastoma (Bone)	0/2
Chondrosarcoma (Bone)	0/3
Metastatic adenocarcinoma (Bone)	0/1
Metastatic carcinoma (Bone)	0/4
Pericardial mesothelial sarcoma (Pericardium)	0/1
Mesothelioma (Peritoneum)	0/1
Carcinosarcoma (Peritoneum)	0/2
Spindle cell sarcoma (Peritoneum)	0/1
Rhabdomyosarcoma (Peritoneum)	0/1
Angioleiomyoma (Soft tissue)	0/1
Alveolar rhabdomyosarcoma (Soft tissue)	2/3
Embryonal rhabdomyosarcoma (Soft tissue)	1/3
Pleomorphic rhabdomyosarcoma (Soft tissue)	0/1
Polymorphic rhabdomyosarcoma (Soft tissue)	3/4
Clear cell sarcoma (Soft tissue)	0/1
Dermatofibrosarcoma protuberans (Soft tissue)	0/4
Epithelioid sarcoma (Soft tissue)	0/3
Fibrolipoma (Soft tissue)	0/1
Fibroma (Soft tissue)	0/2
Fibrosarcoma (Soft tissue)	2/25
Hemangiopericytosarcoma (Soft tissue)	0/1
Lipoma (Soft tissue)	0/1
Liposarcoma (Soft tissue)	0/17
Synovial sarcoma (Soft tissue)	0/4
Malignant fibrous histiocytoma	5/8

Precision

Precision studies for CONFIRM anti-CD68 (KP-1) 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, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT, BenchMark GX, 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.

REFERENCES

1. Chistiakov DA, Killingsworth MC, Myasoedova VA, Orekhov AN, Bobryshev YV. CD68/macrosialin: not just a histochemical marker. Lab Invest. 2017;97(1):4-13.
2. Alessandrini F, Pezze L, Ciribilli Y. LAMPs: Shedding light on cancer biology. Semin Oncol. 2017;44(4):239-253.
3. Barois N, de Saint-Vis B, Lebecque S, Geuze HJ, Kleijmeer MJ. MHC class II compartments in human dendritic cells undergo profound structural changes upon activation. Traffic. 2002;3(12):894-905.
4. Song L, Lee C, Schindler C. Deletion of the murine scavenger receptor CD68. J Lipid Res. 2011;52(8):1542-1550.
5. Favara BE, Feller AC, Pauli M, et al. Contemporary classification of histiocytic disorders. The WHO Committee On Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society. Med Pediatr Oncol. 1997;29(3):157-166.
6. Ferenbach D, Hughes J. Macrophages and dendritic cells: what is the difference? Kidney Int. 2008;74(1):5-7.
7. Dabbs DJ. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications, 5th Edition. 5th ed. Philadelphia, PA: Elsevier; 2019.
8. Tsang WYW, Chan JKC. Kp1 (Cd68) Staining of Granular-Cell Neoplasms - Is Kp1 a Marker for Lysosomes Rather Than the Histiocytic Lineage. Histopathology. 1992;21(1):84-86.
9. Gottfried E, Kunz-Schughart LA, Weber A, et al. Expression of CD68 in non-myeloid cell types. Scand J Immunol. 2008;67(5):453-463.
10. Hameed A, Hruban RH, Gage W, Pettis G, Fox WM, 3rd. Immunohistochemical expression of CD68 antigen in human peripheral blood T cells. Hum Pathol. 1994;25(9):872-876.
11. Emile JF, Abla O, Fraitag S, et al. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. Blood. 2016;127(22):2672-2681.
12. Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM. Clinical Immunology: Principles and Practice. 5th edition ed: Elsevier Limited; 2019.
13. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
14. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
15. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
16. 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 dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
D	Updates to Specimen Preparation, Warnings and Precautions, , Staining Procedure, Analytical Performance, and Symbols, Intellectual Property, and Contact Information sections. Added the Benchmark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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