CD68 [KP1]
Concentrated and Prediluted Monoclonal Antibody
901-033-032620

**Intended Use:**
For In Vitro Diagnostic Use

CD68 [KP1] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CD68 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:**
The CD68 antigen is a 110 kDa highly glycosylated transmembrane protein which is mainly located in lysosomes. Studies have shown the antibody stains macrophages in many human tissues including Kupffer’s cells and macrophages in the red pulp of the spleen, in lung alveoli, in lamina propria of the gut, and in the bone marrow (1). Antigen-presenting cells are either negative or show weak and/or restricted areas of reactivity. Peripheral blood monocytes are also positive with granular staining pattern. The antibody reacts with myeloid precursors and peripheral blood granulocytes (2-5). In addition, the antibody reacts with plasmacytoid T-cells that are present in many reactive lymph nodes, and are believed to be of monocyte/macrophage origin (5). The antibody marks the malignant cells in chronic and acute myeloid leukemia (2). A positive staining of normal and neoplastic mast cells is seen with the antibody, as well as staining of a variable number of cells in malignant melanomas (4). Studies have shown that CD68 (KP1) is formalin-sensitive and false negatives can occur without proper pretreatment.

**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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** KP1

**Isotype:** IgG1/kappa

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

**Epitope/Antigen:** CD68

**Cellular Localization:** Cytoplasmic

**Positive Tissue Control:** Tonsil

**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

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

**Storage and Stability:**
Store at 2ºC to 8ºC. 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 2ºC to 8ºC.

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

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

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

**Digestion Method (Optional):** Digest with Pepsin enzyme for 30-60 seconds at RT.

**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:**

IP033 is intended for use with 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 pretreatment.

**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):**

OAI033 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:** CD68

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

**Dewaxing (DS Option):** DS2

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

**Reagent Name, Time, Temp.:** CD68, 30 min., 25ºC

**Protocol Recommendations (Ventana BenchMark ULTRA):**

AVI033 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 16 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 16 minutes, 37ºC

**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 titer lists 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:

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 (NaN₃) 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) (6)
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. (7)
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:

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