### Intended Use:
CD68 [KP-1] 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 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 monocytic/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

### Total Protein Concentration:
~10 mg/ml. 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 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

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

#### Peroxide Block:
Block for 5 minutes with Biocare's Peroxidized 1.

### Protocol Recommendations (intelliPATH and manual use) Cont'd:

#### Pretreatment Protocol (recommended):

**Pretreatment Protocol:**

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

**Digestion Method (Optional):**

Digest with Pepsin enzyme for 30-60 seconds at RT.

**Protein Block (Optional):**

Incubate for 5-10 minutes at RT with Biocare's 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 Biocare's Wasp Red.

**Counterstain:**

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

### Technical Note:
This antibody has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection and intelliPATH Universal HRP Detection Kit. Other Biocare polymer detection kits may be used; however, users must validate incubation times and protocols for their specific application. Use TBS for washing steps.

### intelliPATH™ Automated Slide Stainer:
IP033 is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxidase block with intelliPATH Peroxidase Blocking Reagent (IPBS000) may be performed following pretreatment.

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

**OAI033 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide StainerEditor 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; 103°C

#### Reagent Name, Time, Temp.:
CD68, 30 min., 25°C

#### Limitations:
The optimum antibody dilution and protocols for 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
Limitations Cont’d:
recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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/.

References:

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.