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antibody and ELISA experts

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Anti-Macrosialin CD68 Antibody

Catalog Number: PA1518

About CD68

CD68 is a heavily glycosylated glycoprotein that is highly expressed in macrophages and other mononuclear phagocytes. Macrosialin, if not glycosylated, has the main sequence that consists of 354 amino acids with an expected molecular weight of 37.4 kD. CD68, which can be located in the cytoplasmic granules of a variety of blood cells and myocytes, can be detected via immunohistochemistry. It's particularly effective as a marker for monocytes, histiocytes, giant cells, Kupffer cells, and osteoclasts, all of which belong to the macrophage lineage. CD68 is expressed in tissue macrophages and to a lesser extent in dendritic cells. CD68 is involved in tissue macrophage phagocytic activities, as well as intracellular lysosomal metabolism and extracellular cell-cell and cell-pathogen interactions. It works by binding to tissue and organ-specific lectins or selectins, enabling macrophage subsets to be directed to specific locations. Macrophages may be able to crawl over selectin-bearing substrates or other cells if CD68 is rapidly recirculated from endosomes and lysosomes to the plasma membrane.

Overview

Product Name	Anti-Macrosialin CD68 Antibody
Reactive Species	Mouse, Rat
Description	**Superstar antibody:** Boster's anti mouse CD68 antibody (PA1518) is among its top 10 bestselling antibodies, and one of the most cited mouse/rat CD68 antibodies on the market. Because of this antibody's high demand, we have subsequently developed a rabbit monoclonal anti CD68 antibody (M00602-1) targetting a similar epitope. **High specificity:** PA1518 reacts with murine macrosialin/CD68, and has been validated with Western blotting and confirmed its stellar specificity. CD68 is a highly glycosylated protein, and its expected western blot molecular weight is between 80kDa to 110kDa, depending on glycosylation level. PA1518's observed MW in WB is near 90-100kDa. Testing on negative control tissues showed no significant bands (images available on request) or staining. See more info in the positive and negative control design section. Based on immunogen sequence homology, this antibody is not expected to cross react with other proteins from the LAMP family, which complies with QC testing observations. **Great for CD68 IHC:** This antibody produces clean and specific immunobiological stains in both mouse and rat tissues. Our QC team has validated it on spleen and liver tissues of both mice and rats. Click product images for more details on experiment conditions. **More about CD68:** CD68 is a cell surface marker often used to identify macrophages and other cell types in the monocyte lineage. It is a transmembrane protein that binds to electins and selectins and plays a role in macrophage homing movement. Check out the CD68 biomarker page for more information on CD68 and view all CD68 antibodies, ELISA kits and proteins.

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Application	Flow Cytometry, IF, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P31996

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence in the middle region of mouse CD68, different from the related rat sequence by one amino acid.
Predicted Reactive Species	Hamster
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Mouse, Rat, By Heat Immunofluorescence, 5ug/ml, Mouse Flow Cytometry(Fixed), 1-3 ug/1x10^6 cells, Mouse

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Anti-Macrosialin CD68 Antibody (PA1518) Images

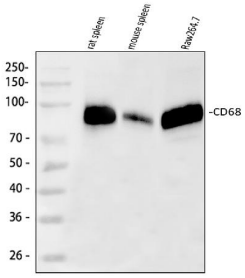

Figure 1. Western blot analysis of CD68 using anti-CD68 antibody (PA1518).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: rat spleen tissue lysates,
Lane 2: mouse spleen tissue lysates,
Lane 3: mouse RAW264.7 whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CD68 antigen affinity purified polyclonal antibody (Catalog # PA1518) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CD68 at approximately 90-100 kDa. The expected band size for CD68 is at 37 kDa.

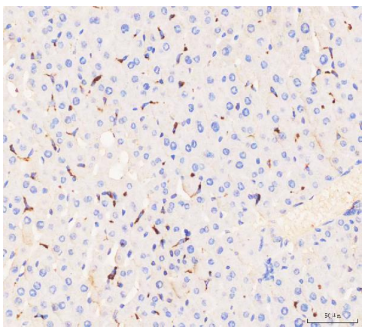

Figure 2. IHC analysis of CD68 using anti-CD68 antibody (PA1518).
CD68 was detected in a paraffin-embedded section of mouse liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

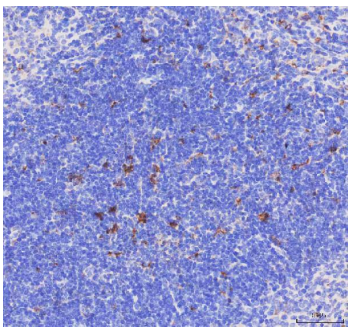

Figure 3. IHC analysis of CD68 using anti-CD68 antibody (PA1518).
CD68 was detected in a paraffin-embedded section of mouse spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

Figure 4. IHC analysis of CD68 using anti-CD68 antibody

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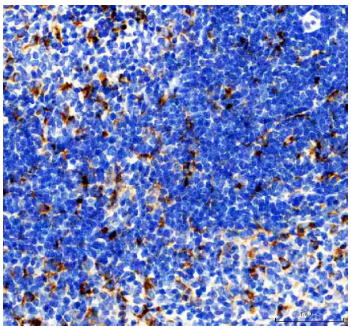

(PA1518).
CD68 was detected in a paraffin-embedded section of rat spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

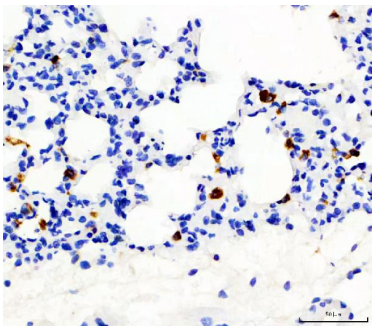

Figure 5. IHC analysis of CD68 using anti-CD68 antibody (PA1518).
CD68 was detected in a paraffin-embedded section of rat lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

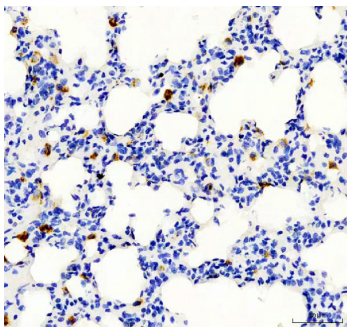

Figure 6. IHC analysis of CD68 using anti-CD68 antibody (PA1518).
CD68 was detected in a paraffin-embedded section of rat lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

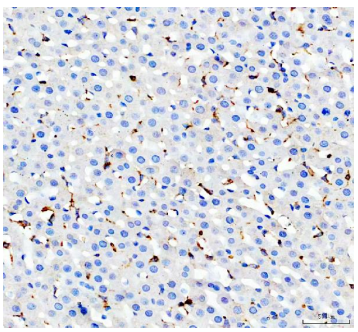

Figure 7. IHC analysis of CD68 using anti-CD68 antibody (PA1518).
CD68 was detected in a paraffin-embedded section of rat liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

Figure 8. IF analysis of CD68 using anti-CD68 antibody (PA1518).
CD68 was detected in a paraffin-embedded section of mouse spleen tissue. Heat mediated antigen retrieval was

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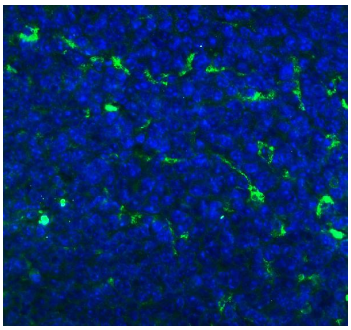

performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 ug/mL rabbit anti-CD68 Antibody (PA1518) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127)) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

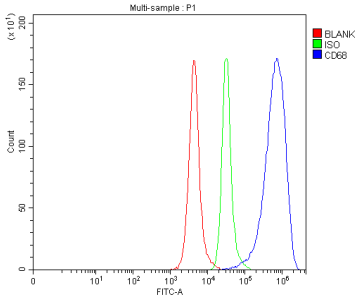

Figure 9. Flow Cytometry analysis of RAW264.7 cells using anti-CD68 antibody (PA1518).
Overlay histogram showing RAW264.7 cells stained with PA1518 (Blue line). The cells were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-CD68 Antibody (PA1518, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

56 Publications Citing This Product

1. PubMed ID: 10.1158/1535-7163.MCT-15-0995,

HPMA–Copolymer Nanocarrier Targets Tumor-Associated Macrophages in Primary and Metastatic Breast Cancer

The immunofluorescence detection of the CD68 was performed on tumor specimens using the Discovery XT processor to confirm the co-staining of macrophages with the HPMA copolymer P-Alexa647-FA. Using IF and confocal microscopy the P-Alexa647-FA signal within TAMs costained with CD68 from both primary and metastatic tumor tissue specimens.

2. PubMed ID: 28854169,

A somatic mutation in erythro-myeloid progenitors causes neurodegenerative disease

Immunostaining for CD68, IBA1, YFP and phosphorylated (p)ERK demonstrated that microglial clusters represented the accumulation of EMP-derived (YFP+) pERK+ microglia. The pathophysiological consequences of a somatic BRAF(V600E) mutation in different haematopoietic lineages are markedly distinct. BRAF(V600E) expression in HSCs can cause a tumoral disease, but results in a neurodegenerative inflammatory disorder when expressed in EMP, thereby linking the pathophysiology of clonal and neurodegenerative disorders.

3. PubMed ID: 31327655, Su W, Han HH, Wang Y, Zhang B, Zhou B, Cheng Y, Rumandla A, Gurrapu S, Chakraborty G, Su J, Yang G, Liang X, Wang G, Rosen N, Scher HI, Ouerfelli O, Giancotti FG. The Polycomb Repressor Complex 1 Drives Double-Negative Prostate Cancer Metastasis by Coordinating Stemness and Immune Suppression. Cancer Cell. 2019 Aug 12;36(2):139-155.e10. doi: 10.1016/j.ccell.2019.06.009. Epub 2019 Jul 18. PMID: 31327655; PMCID: PMC7210785.

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