abcam

Product datasheet

Anti-CD68 antibody [EPR20545] - BSA and Azide free ab227458

Recombinant RabMAb

14 Images

Overview

Product name	Anti-CD68 antibody [EPR20545] - BSA and Azide free
Description	Rabbit monoclonal [EPR20545] to CD68 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, mIHC
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human tonsil, fetal liver and fetal spleen lysates; THP-1 and U937 whole cell lysates. IHC-P: Human tonsil, liver and cervix carcinoma. mIHC: Human liver tissue, human duodenum tissue, human colon tissue. ICC/IF: THP-1 and U937 cells.
General notes	ab227458 is the carrier-free version of **ab213363**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2

	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20545
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227458 in the following tested applications.

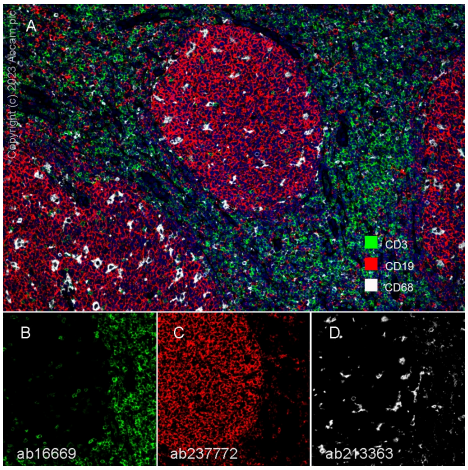
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 37 kDa).
ICC/IF		Use at an assay dependent concentration.
mIHC		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function	Could play a role in phagocytic activities of tissue macrophages, both in intracellular lysosomal metabolism and extracellular cell-cell and cell-pathogen interactions. Binds to tissue- and organ-specific lectins or selectins, allowing homing of macrophage subsets to particular sites. Rapid recirculation of CD68 from endosomes and lysosomes to the plasma membrane may allow macrophages to crawl over selectin-bearing substrates or other cells.
Tissue specificity	Highly expressed by blood monocytes and tissue macrophages. Also expressed in lymphocytes, fibroblasts and endothelial cells. Expressed in many tumor cell lines which could allow them to attach to selectins on vascular endothelium, facilitating their dissemination to secondary sites.
Sequence similarities	Belongs to the LAMP family.
Post-translational modifications	N- and O-glycosylated.
Cellular localization	Cell membrane and Endosome membrane. Lysosome membrane.

Images



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

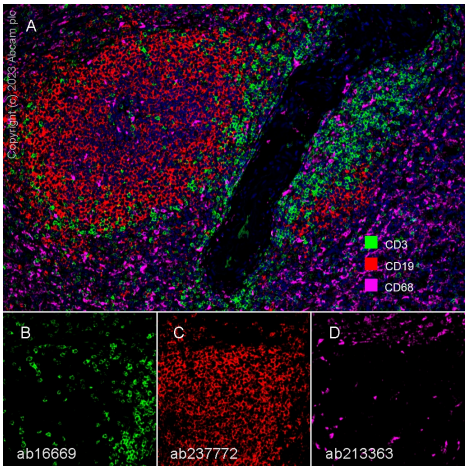
This data was developed using **ab213363**, the same antibody clone in a different buffer formulation.

Panel A: merged staining of anti-CD68 (gray; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, and counterstaining was with DAPI.

Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD68 stained on macrophages with **ab213363** 1/500 dilution

The section was incubated in three rounds of staining: in the order of **ab213363** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

This data was developed using **ab213363**, the same antibody clone in a different buffer formulation.

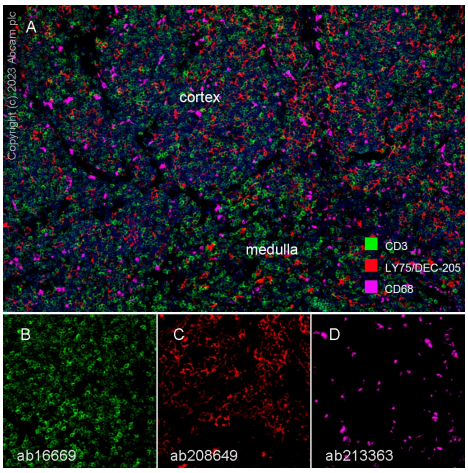
Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, and counterstaining was with DAPI.

Panel B: anti-CD3 stained on T cells with **ab16669** at 1/500 dilution
Panel C: anti-CD19 stained on B cells with **ab237772** at 1/5000 dilution
Panel D: anti-CD68 stained on macrophages with **ab213363** 1/500 dilution

The section was incubated in three rounds of staining: in the order of **ab213363** and **ab16669** for 30 mins, then **ab237772** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval

solution2) for 20 mins.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

This data was developed using **ab213363**, the same antibody clone in a different buffer formulation.

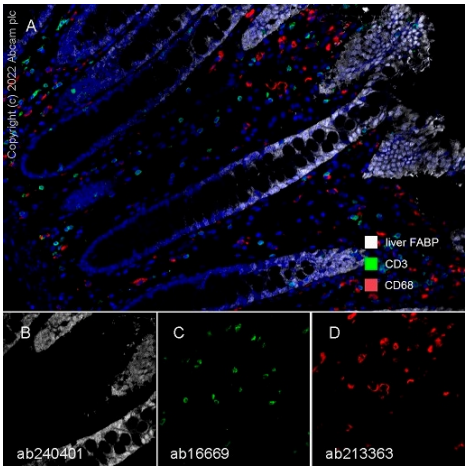
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human thymus tissue labeling CD3 with **ab16669** at 1/500 dilution, LY75/DEC-205 with **ab208649** at 1/15000, and CD68 with **ab213363** at 1/500 dilution.

Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 (green; Opal™520) and anti-LY75/DEC-205 (red; Opal™570) on human thymus.
Panel B: anti-CD3 stained on T cells.
Panel C: anti-LY75/DEC-205 stained on thymic cortical epithelium and dendritic cells.
Panel D: anti-CD68 stained on macrophages.

Sections were treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins before antibody incubation. The section was incubated in three rounds of staining: in the order of **ab213363**, **ab16669**, and **ab208649** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

DAPI was used as a nuclear counterstain.

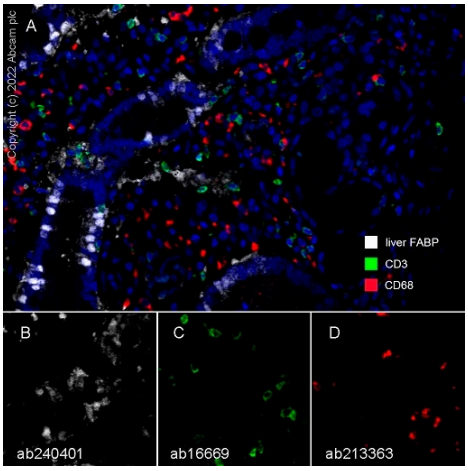
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Fluorescence multiplex immunohistochemical analysis of the human colon (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (**ab240401**, gray; Opal™690), anti-CD3 (**ab16669**, green; Opal™520) and anti-CD68 (**ab213363**, red; Opal™570) on human colon. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-CD3 stained on T cells. Panel D: anti-CD68 stained on macrophages. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of **ab240401** (1/8000 dilution), **ab16669** (1/150 dilution), and **ab213363** (1/500 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

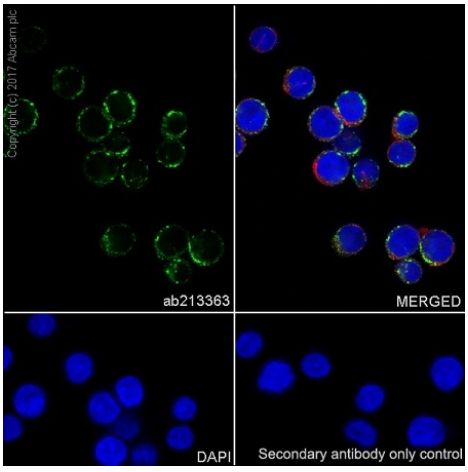
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213363**).



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Fluorescence multiplex immunohistochemical analysis of the human duodenum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-liver FABP (**ab240401**, gray; Opal™690), anti-CD3 (**ab16669**, green; Opal™520) and anti-CD68 (**ab213363**, red; Opal™570) on human duodenum. Panel B: anti-liver FABP stained on enterocytes. Panel C: anti-CD3 stained on T cells. Panel D: anti-CD68 stained on macrophages. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of **ab240401** (1/8000 dilution), **ab16669** (1/150 dilution), and **ab213363** (1/500 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213363**).



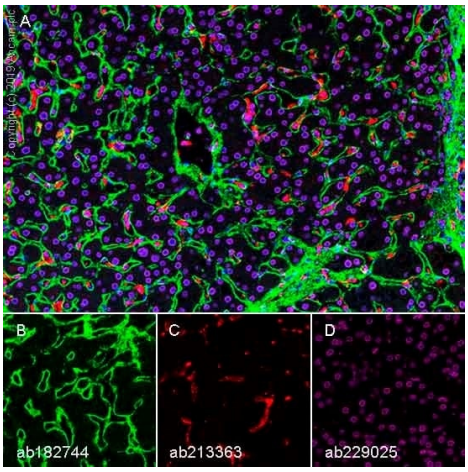
Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Immunofluorescent analysis of 100% methanol-fixed THP-1 (human monocytic leukemia cell line) cells labeling CD68 with **ab213363** at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on THP-1 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213363**).



Multiplex immunohistochemistry - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

This data was developed using the same antibody clone in a different buffer formulation (**ab213363**).

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue.

Panel A: Merged staining of Collagen VI (**ab182744**; green), anti-CD68 (**ab213363**; red) and anti-Lamin B1 (**ab229025**; magenta).

Panel B: Anti-Collagen VI (green) stained on extracellular matrix.

Panel C: Anti-CD68 (red) stained on Kupffer cells.

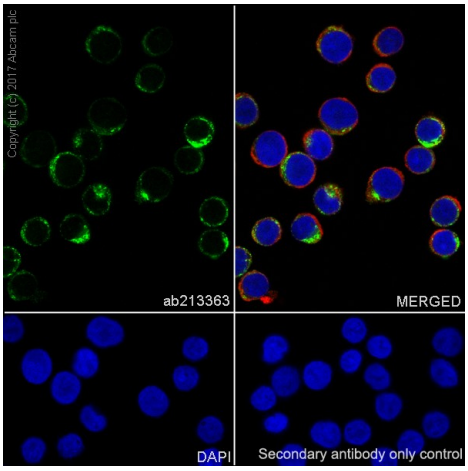
Panel D: Anti-Lamin B1 (magenta) stained on nuclear envelope.

Key protocol steps: The section was incubated in three rounds of staining with **ab182744** (1/1000 dilution), **ab213363** (1/1000 dilution) and **ab229025** (1/4000 dilution) for 30 mins at room temperature. Each round was followed by tyramide signal amplification with the appropriate fluorophore. Heat mediated antigen retrieval was used (Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins after every round of antibody/fluorophore staining.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

DAPI was used as a nuclear counter stain. A ready-to-use anti-Rabbit and Mouse Polymer HRP was used as a secondary.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



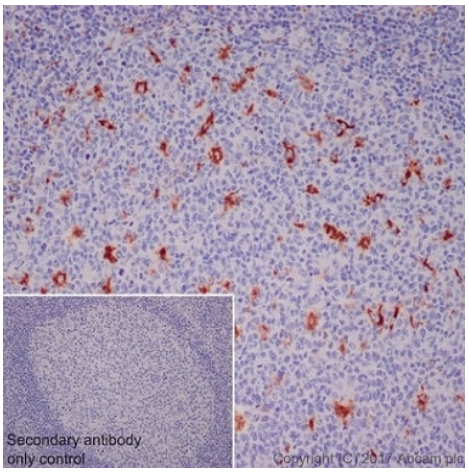
Immunocytochemistry/ Immunofluorescence - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Immunofluorescent analysis of 100% methanol-fixed U937 (human histiocytic lymphoma cell line) cells labeling CD68 with **ab213363** at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on U937 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213363**).



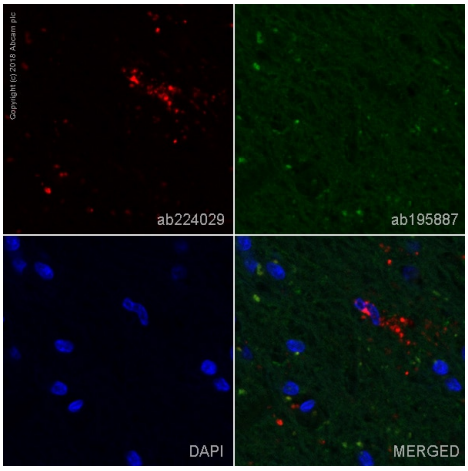
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue, labeling CD68 with **ab213363** at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on macrophages of human tonsil is observed (PMID: 19543531). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213363**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

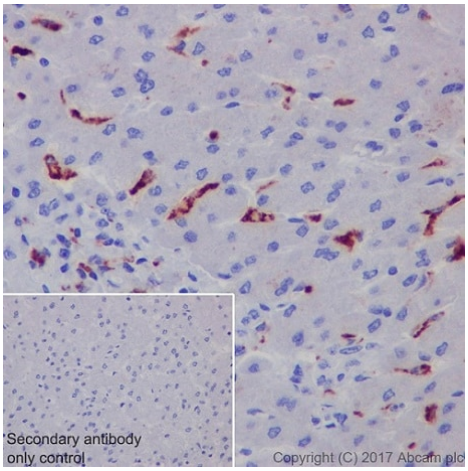
Clone EPR20545 (ab227458) has been successfully conjugated by Abcam. This image was generated using Anti-CD68 antibody [EPR20545] (Alexa Fluor® 647). Please refer to **ab224029** for protocol details.

IHC image of CD68 staining in a section of formalin-fixed paraffin-embedded human prefrontal cortex tissue.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6) in a Biocare Medical NxGen pressure cooker using retrieval settings of 110^{O}C for 20 minutes. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with **ab224029** at 1/1000 dilution (shown in red) and counterstained using **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labeled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



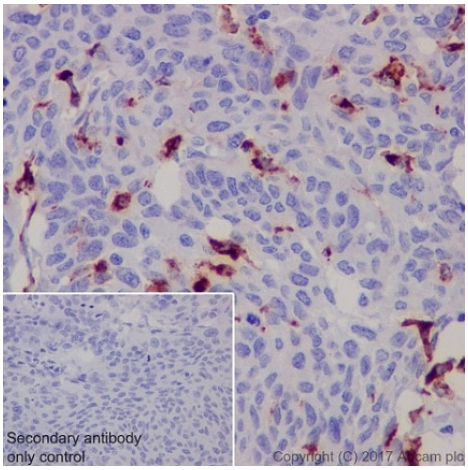
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling CD68 with **ab213363** at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on Kupffer cells of human liver is observed (PMID: 12118106). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213363**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

This IHC data was generated using the same anti-CD68 antibody clone, EPR20545, in a different buffer formulation (cat# **ab213363**).

Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue labeling CD68 with **ab213363** at 1/8000 dilution, followed by Goat anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on macrophages of human cervical carcinoma is observed (PMID: 12118106). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-Rabbit IgG H&L (HRP) Ready to use.

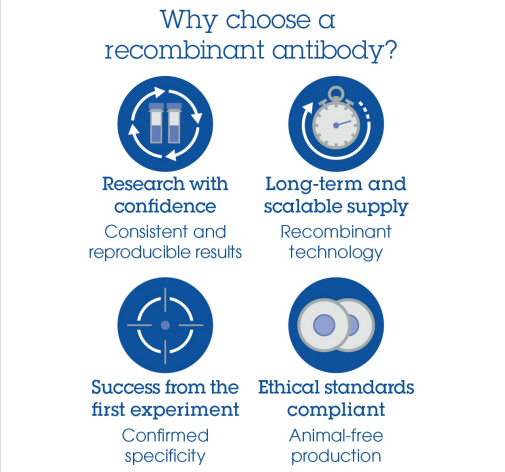
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Tissue Microarray (TMA) data for ab213363

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x (immune cells ✓)	Human placenta	x (immune cells ✓)	Clear cell carcinoma of human kidney	x (immune cells ✓)	Human gastric adenocarcinoma	x (immune cells ✓)
Human cerebrum	x	Human skeletal muscle	x	Human astrocytoma	x (immune cells ✓)	Human hepatocellular carcinoma	x (immune cells ✓)
Human colon	x (immune cells ✓)	Human skin	x (immune cells ✓)	Human bladder cancer	x (immune cells ✓)	Human lung carcinoma	x (immune cells ✓)
Human endometrium	x (immune cells ✓)	Human spleen	✓	Human breast carcinoma	x (immune cells ✓)	Human ovarian carcinoma	x (immune cells ✓)
Human kidney	x	Human stomach	x (immune cells ✓)	Human cervical carcinoma	x (immune cells ✓)	Human pancreatic carcinoma	x (immune cells ✓)
Human liver	x (Kupffer cells ✓)	Human testis	x (immune cells ✓)	Human colon carcinoma	x (immune cells ✓)	Human prostatic hyperplasia	x
Human lung	x (immune cells ✓)	Human thyroid	x (immune cells ✓)	Human endometrial carcinoma	x (immune cells ✓)	Human thyroid carcinoma	x (immune cells ✓)
Human mammary gland	x (immune cells ✓)	Human tonsil	✓				
Human pancreas	x (immune cells ✓)						

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

Tissue Microarrays stained for " Anti-CD68 antibody [EPR20545]" using " **ab213363**" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). The sections were incubated with **ab213363** at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Anti-CD68 antibody [EPR20545] - BSA and Azide free (ab227458)

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