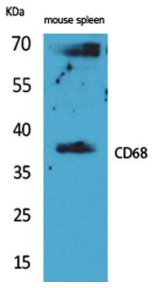
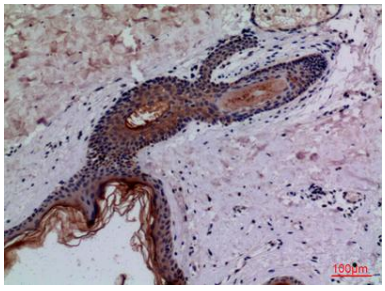
Product Data Sheet:
CD68 Rabbit Polyclonal Antibody

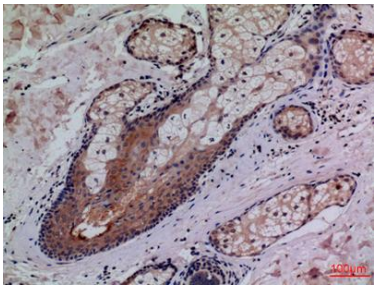
Cat. No:	AB-84755
Conjugate:	Unconjugated
Size:	100 ug
Clone:	POLY
Concentration:	1mg/ml
Host:	Rabbit
Isotype:	IgG
Immunogen:	The antiserum was produced against synthesized peptide derived from the Internal region of human CD68. AA range:171-220.
Reactivity:	Human
Applications:	Western Blot: 1/500 – 1/2000. Immunohostochemistry (paraffin-embedded tissues) 1:100-300 Immunofluorescence ELISA: 1/20000.
Molecular Weight:	37kD
Purification:	The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.
Synonyms:	CD68; Macrosialin; Gp110; CD68
Background:	This gene encodes a 110-kD transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. It is a member of the lysosomal/endosomal-associated membrane glycoprotein (LAMP) family. The protein primarily localizes to lysosomes and endosomes with a smaller fraction circulating to the cell surface. It is a type I integral membrane protein with a heavily glycosylated extracellular domain and binds to tissue- and organ-specific lectins or selectins. The protein is also a member of the scavenger receptor family. Scavenger receptors typically function to clear cellular debris, promote phagocytosis, and mediate the recruitment and activation of macrophages. Alternative splicing results in multiple transcripts encoding different isoforms.
Form:	Liquid
Buffer:	Liquid in PBS containing 50% glycerol, 0.5% BSA and 0.02% sodium azide.
Storage:	Store at -20°C. Avoid repeated freeze-thaw cycles.



Western Blot analysis of mouse spleen cells using CD68 Polyclonal Antibody.



Immunohistochemical analysis of paraffin-embedded human-skin,

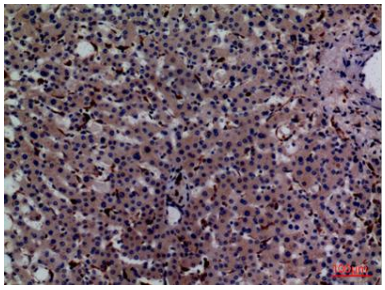


Immunohistochemical analysis of paraffin-embedded human-skin,

Secondary antibody was diluted at 1:20000

antibody was diluted at 1:100.

antibody was diluted at 1:100.



Immunohistochemical analysis of paraffin-embedded human-liver, antibody was diluted at 1:100.

Protocol:

Paraffin section staining steps:
1. Dewax the paraffin sections to water: put the sections into ① xylene for 15min-② xylene for 15min-③ anhydrous ethanol for 5min-④ anhydrous ethanol for 5min-⑤ 90% alcohol for 5min-⑥ 75% alcohol for 5min, and wash with tap water.
2. Wash with pure water, place the sections in 0.01M citrate buffer (PH6.0), heat in a microwave oven for antigen repair, and repair at medium-high heat for 2~8min. The specific time needs to be adjusted according to the actual situation of the sample.
3. Cool to room temperature, draw circles around the tissue with a tissue pen to prevent the incubation solution from flowing away in the subsequent process, and wash with PBS.
4. Incubate at room temperature with 3% H2O2 for 15~30min to block endogenous peroxidase, and wash with PBS 3 times, each time for 5min.
5. Dilute the antibody with 5% BSA in a certain proportion, add an appropriate amount of primary antibody (see Appendix 1) working solution, and incubate at 4℃ overnight.
6. Rewarm, wash with PBS 3 times, each time for 5min.
7. Add the secondary antibody (see Appendix 2) working solution, incubate in a 37℃ water bath for 30min, and wash with PBS 3 times, 5min each time.
8. Prepare DAB working solution, control the color development under a microscope, positive is brown-yellow, and tap water washing can stop the color development.
9. Wash with tap water, stain the nucleus with hematoxylin for 1~5min, differentiate with 1% hydrochloric acid, wash with tap water, turn blue with 1% ammonia water, wash with tap water. If the nuclear staining effect is not good, use lapis lazuli blue dye for 3~5min, wash with tap water and then stain with hematoxylin.
10. Dehydrate and seal: Put the slices into ①75% alcohol for 5min -②anhydrous ethanol for 5min -③anhydrous ethanol for 5min -④xylene for 5min-⑤xylene for 5min-⑥xylene for 5min, dehydrate and make it transparent, and seal with neutral gum.

Steps for staining frozen sections:
1. Take out the frozen sections from -20℃, fix them with 4% paraformaldehyde for 2 minutes, wash with pure water, and rewarm.
Steps 2 to 10 are the same as paraffin sections. (Frozen sections are easy to fall off, so they do not need to be repaired)

Cell staining step:
1. Pour off the culture medium and wash with PBS 3 times, 5 minutes each time.
2. Fix with 4% paraformaldehyde for 20 minutes, wash with PBS 3 times, 5 minutes each time.
3. Draw circles with a histochemical pen to prevent the incubation solution from flowing away in the following process, and wash with PBS.
4. Dilute the antibody with 5% BSA in a certain proportion, add an appropriate amount of primary antibody (see Appendix 1) working solution, and incubate at 4℃ overnight.
5. Rewarm, wash with PBS 3 times, 5 minutes each time.
6. Add the secondary antibody (see Appendix 2) working solution, incubate in a 37℃ water bath for 30 minutes, and wash with PBS 3 times, 5 minutes each time.

7. Prepare DAB working solution, control the color development under a microscope, the positive is brown-yellow, and the color development can be stopped by washing with tap water.
8. Wash with tap water, stain the nucleus with hematoxylin for 1~5min, differentiate with 1% hydrochloric acid, wash with tap water, turn blue with 1% ammonia water, wash with tap water. If the nuclear staining effect is not good, use lapis lazuli blue dye for 3~5min, wash with tap water and then stain with hematoxylin.
9. Take out the slide, dehydrate with alcohol xylene to make it transparent, seal with neutral gum or do not dehydrate, and seal with glycerol gelatin.
Note: Suspended cells need to be centrifuged, vortexed, and smeared before staining. (Remove the upper liquid after centrifugation, wash 3 times with PBS, fix with 4% paraformaldehyde for 20min, wash 3 times with PBS, vortex and mix well before smearing, pay attention to the cell density, dry at room temperature or dry for subsequent staining, and be careful not to wash too much, as it is easy to fall off.)