abcam

ab325777

Anti-CD66b antibody [RM1323]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM1323
Purification technique	Affinity purification Protein A
Specificity	This antibody is specific to CEACAM8/CD66 and shows no cross-reactivity with CEACAM1/CD66a, CEACAM3/CD66d, CEACAM5/CD66e, CEACAM6/CD66c.
Concentration	0.488 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Transfected cell lysate - Human
Dilution info	1/1000

Notes	-

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Transfected cell line - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Transfected cell line - Human
Dilution info	1/20000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/20000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human

Dilution info	1/50
Notes	-

Not recommended

Species	Transfected cell lysate - Human, Transfected cell line - Human
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/5000
Notes	-

Not recommended

Species	Transfected cell lysate - Human, Transfected cell line - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Transfected cell lysate - Human, Transfected cell line - Human
Dilution info	-
Notes	-

Target data

See full target information CEACAM8

Function	Cell surface glycoprotein that plays a role in cell adhesion in a calcium-independent manner (PubMed:11590190, PubMed:2022629, PubMed:8776764). Mediates heterophilic cell adhesion with other carcinoembryonic antigen-related cell adhesion molecules, such as CEACAM6 (PubMed:11590190, PubMed:2022629, PubMed:8776764). Heterophilic interaction with CEACAM8 occurs in activated neutrophils (PubMed:8776764).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

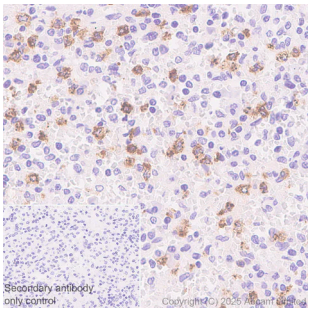
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

12 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human spleen using rabbit Anti-CD66b antibody

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling CD66b with ab325777 at 1/20000 (0.025 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human spleen.

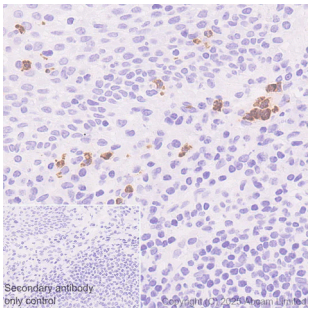
The primary antibody was incubated for 30 mins at room temperature.

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

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-CD66b antibody

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling CD66b with ab325777 at 1/20000 (0.025 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human tonsil.

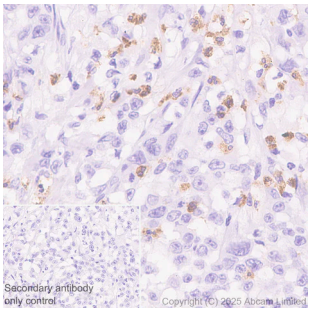
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human gastric cancer using rabbit Anti-CD66b antibody

Immunohistochemical analysis of paraffin-embedded Human gastric cancer tissue labeling CD66b with ab325777 at 1/20000 (0.025 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on immune cells of human gastric cancer.

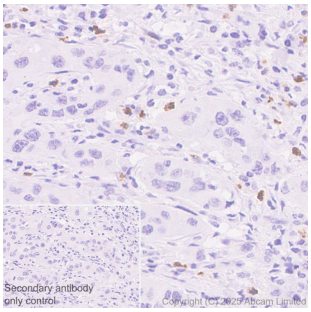
The primary antibody was incubated for 30 mins at room temperature.

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

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human cervical cancer using rabbit Anti-CD66b antibody

Immunohistochemical analysis of paraffin-embedded Human cervical cancer tissue labeling CD66b with ab325777 at 1/20000 (0.025 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on immune cells of human cervical cancer.

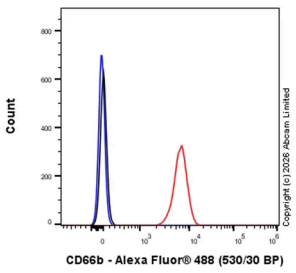
The primary antibody was incubated for 30 mins at room temperature.

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

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



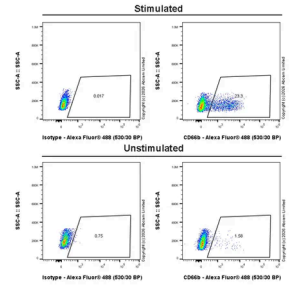
Flow Cytometry - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Flow Cytometry staining of Human neutrophils using rabbit Anti-CD66b antibody

Flow cytometric analysis of Human neutrophils cells labelling CD66b with ab325777 at 1/5000 (0.01ug) / Red compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Gated on viable cells.



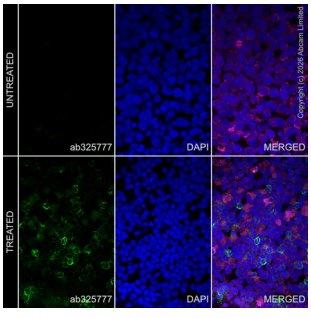
Flow Cytometry - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Flow Cytometry staining using rabbit Anti-CD66b antibody

Flow cytometric analysis of Unstimulated HL-60 (Human acute promyelocytic leukemia promyeloblast) (Lower) HL-60 stimulated with 1.25% DMSO for 5 days (Upper) cells labelling CD66b with ab325777 at 1/5000 dilution (0.01ug) / Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Gated on viable cells.



Immunocytochemistry/ Immunofluorescence - Anti-CD66b antibody [RM1323] (ab325777)

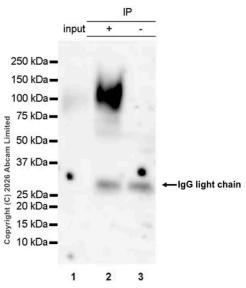
CD66b Immunocytochemistry/ Immunofluorescence staining of HL-60 cells treated with DMSO (1.25%) for 5 days using rabbit Anti-CD66b antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HL-60 (human acute promyelocytic leukemia promyeloblast) HL-60 cells treated with DMSO (1.25%) for 5 days cells labelling CD66b with ab325777 at 1/50 (9.94 µg/ml) dilution , followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Confocal image showing increased cytoplasmic staining in differentiated HL-60 cells (treatment with DMSO (1.25%) for 5 days), (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab7291 Anti-alpha Tubulin mouse monoclonal antibody was used to counterstain tubulin at 1/1000 dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Immunoprecipitation - Anti-CD66b antibody [RM1323] (ab325777)

CD66b was immunoprecipitated from 0.35 mg HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate with ab325777 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab325777 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate
Lane 2: ab325777 IP in HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab325777 in HL-60 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 93 seconds.

All lanes:
Immunoprecipitation - Anti-CD66b antibody [RM1323] (ab325777) at 1/1000 dilution

Lane 1:
HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate at 10 µg

Lane 2:
ab325777 at 1/30 IP in HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate

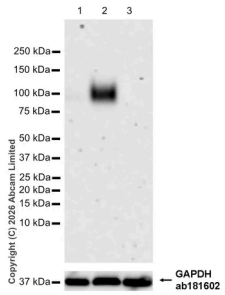
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab325777 in HL-60 whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 95 kDa

Exposure time: 93s



Western blot - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Western blot staining using rabbit Anti-CD66b antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Low expression: liver and cerebellum.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-CD66b antibody [RM1323] (ab325777) at 1/1000 dilution

Lane 1:
Human liver tissue lysate at 20 µg

Lane 2:
human spleen tissue lysate at 20 µg

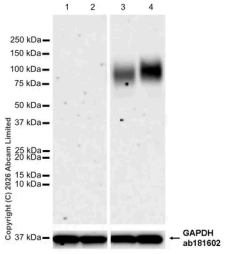
Lane 3:
Human cerebellum tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 95 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Western blot staining using rabbit Anti-CD66b antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: SW1463 and HCC827.

This blot was developed using a high-sensitivity ECL substrate, allowing for the detection of proteins in the mid-femtogram range.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-CD66b antibody [RM1323] (ab325777) at 1/1000 dilution

Lane 1:
SW1463 (human Large intestine Rectum epithelial) whole cell lysate at 20 µg

Lane 2:
HCC827 (human lung adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
Untreated HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate at 20 µg

Lane 4:
HL-60 treated with 1.25% Dimethyl sulfoxide (DMSO) for 5 days whole cell lysate at 20 µg

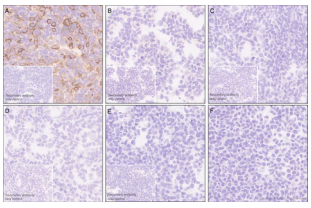
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 95 kDa, 36 kDa

Exposure time: 180s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD66b antibody

Immunohistochemical analysis of paraffin-embedded (A): HEK-293T cells transfected with a CEACAM8 expression vector containing a his tag. (B): HEK-293T cells transfected with a CEACAM1 expression vector containing a his tag. (C): HEK-293T cells transfected with a CEACAM3 expression vector containing a his tag. (D): HEK-293T cells transfected with a CEACAM5 expression vector containing a his tag. (E): HEK-293T cells transfected with a CEACAM6 expression vector containing a his tag. (F): HEK-293T cells transfected with empty vector containing a his tag. tissue labeling CD66b with ab325777 at 1/20000 (0.025 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Positive staining on (A) HEK-293T cells transfected with a CEACAM8 expression vector containing a his tag. No staining on HEK-293T cells transfected with a CEACAM1 (B), CEACAM3 (C), CEACAM5 (D), CEACAM6 (E), and empty (F) expression vector containing a his tag.

This antibody does not cross-react with CEACAM1, CEACAM3, CEACAM5 and CEACAM6 proteins.

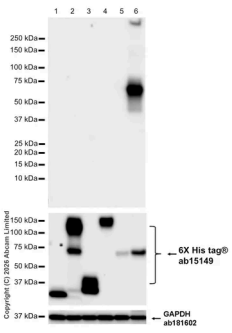
The primary antibody was incubated for 30 mins at room temperature.

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

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-CD66b antibody [RM1323] (ab325777)

CD66b Western blot staining using rabbit Anti-CD66b antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa);Anti-6X His tag® antibody [AD1.1.10] (ab15149) (27-150KDa).

All lanes:
Western blot - Anti-CD66b antibody [RM1323] (ab325777) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with an empty vector containing a His-tag, whole cell lysate at 5 µg

Lane 2:
293T cells transfected with a human CEACAM1/CD66a expression vector containing a His-tag, whole cell lysate at 5 µg

Lane 3:
293T cells transfected with a human CEACAM3/CD66d expression vector containing a His-tag, whole cell lysate at 5 µg

Lane 4:
293T cells transfected with a human CEACAM5/CD66e expression vector containing a His-tag, whole cell lysate at 5 µg

Lane 5:
293T cells transfected with a human CEACAM6/CD66c expression vector containing a His-tag, whole cell lysate at 5 µg

Lane 6:
293T cells transfected with a human CEACAM8/CD66b expression vector containing a His-tag, whole cell lysate at 5 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 95 kDa, 36 kDa, 27-150 kDa

Exposure time: 15s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.