abcam

ab323612

Anti-HHLA2 antibody [RM2075]

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
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM2075
Purification technique	Affinity purification Protein A
Concentration	0.505 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Transfected cell line - Human
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/500
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat, Transfected cell line - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Transfected cell line - Human, Mouse, Rat

Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Transfected cell line - Human, Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/5000
Notes	-

Not recommended

Species	Transfected cell line - Human, Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information HHLA2

Function	Through interaction with TMIGD2, costimulates T-cells in the context of TCR-mediated activation. Enhances T-cell proliferation and cytokine production via an AKT-dependent signaling cascade.

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.

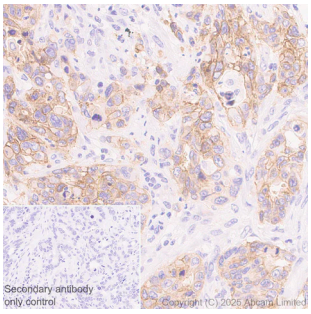
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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9 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon carcinoma tissue using rabbit Anti-HHLA2 antibody

Immunohistochemical analysis of paraffin-embedded Human colon carcinoma tissue labeling HHLA2 with ab323612 at 1/500 (1.01 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

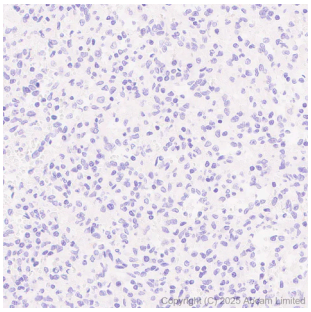
Positive staining on human colon carcinoma. The section was incubated with ab323612 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

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

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human spleen tissue using rabbit Anti-HHLA2 antibody

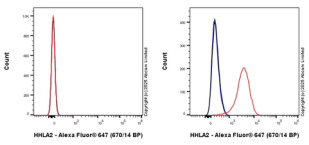
Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling HHLA2 with ab323612 at 1/500 (1.01 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: no staining on human spleen. The section was incubated with ab323612 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



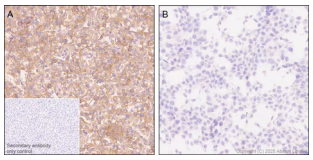
Flow Cytometry - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Flow Cytometry staining using rabbit Anti-HHLA2 antibody

Flow cytometric analysis of Jurkat (human T cell leukemia T lymphocyte from peripheral blood, Left) /A-204 (human muscle rhabdomyosarcoma cell, Right) cells labelling HHLA2 with ab323612 at 1/5000 dilution (0.01 ug) / Right 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® 647, ab150083) at 1/5000 dilution was used as the secondary antibody.

Negative control: Jurkat (PMID: 25549724).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HHLA2 antibody

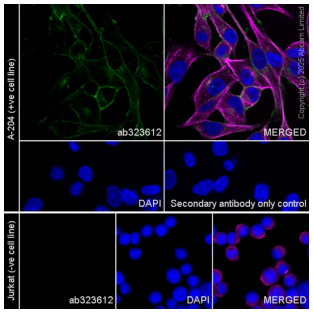
Immunohistochemical analysis of paraffin-embedded (A) A-204 (human muscle rhabdomyosarcoma cell) cell pellet (B) HeLa (human cervical adenocarcinoma epithelial cell) cell pellet labeling HHLA2 with ab323612 at 1/500 (1.01 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on A-204 (image A). No staining on HeLa (image B). The section was incubated with ab323612 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



Immunocytochemistry/ Immunofluorescence - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HHLA2 antibody

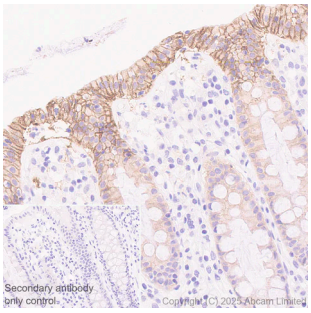
Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized A-204 (human muscle rhabdomyosarcoma cell) cells labelling HHLA2 with ab323612 at 1/50 (10.1 ug/ml)

dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green).

Confocal image showing mainly membranous staining in A-204 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Negative control: Jurkat (PMID: 25549724).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml 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 2ug/ml dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon tissue using rabbit Anti-HHLA2 antibody

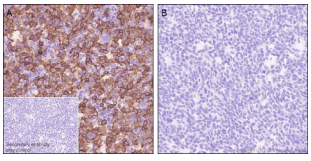
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling HHLA2 with ab323612 at 1/500 (1.01 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human colon. The section was incubated with ab323612 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-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HHLA2 antibody

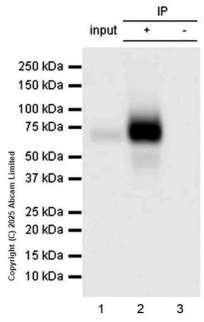
Immunohistochemical analysis of paraffin-embedded (A) HEK-293T (human embryonic kidney epithelial cell) transfected with a human HHLA2 expression vector containing a Myc-His tag. (B) HEK-293T transfected with empty vector containing a Myc-His tag. tissue labeling HHLA2 with ab323612 at 1/2000 (0.253 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on HEK-293T transfected with a Myc-His-tagged human HHLA2 construct (image A). No staining on HEK-293T transfected with empty plasmid (image B). The section was incubated with ab323612 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



Immunoprecipitation - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 was immunoprecipitated from 0.35 mg A-204 (human muscle rhabdomyosarcoma cell) whole cell lysate with ab323612 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab323612 at 1/1000 dilution.

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

All lanes:
Immunoprecipitation - Anti-HHLA2 antibody [RM2075] (ab323612) at 1/1000 dilution

Lane 1:
(Input) A-204 (human muscle rhabdomyosarcoma cell) whole cell lysate at 10 µg

Lane 2:
ab323612 at 1/30 IP in A-204 (human muscle rhabdomyosarcoma cell) whole cell lysate at 10 µg

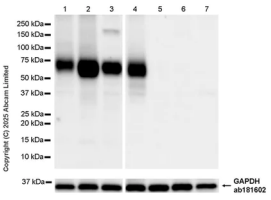
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab323612 in A-204 whole cell lysate at 10 µg

Secondary

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

Observed band size: 70-80 kDa

Exposure time: 6s



Western blot - Anti-HHLA2 antibody [RM2075] (ab323612)

HHLA2 Western blot staining using rabbit Anti-HHLA2 antibody

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

Negative control: HeLa, Jurkat (PMID: 25549724), HepG2.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

Exposure time: Lanes 1-3: 26 seconds; Lanes 4-7: 6 seconds.

All lanes:
Western blot - Anti-HHLA2 antibody [RM2075] (ab323612) at 1/1000 dilution

Lane 1:
AsPC-1 (human Pancreas epithelial cell) whole cell lysate at 20 µg

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

Lane 3:
HDLM-2 (human hodgkin lymphoma cell) whole cell lysate at 20 µg

Lane 4:

A-204 (human muscle rhabdomyosarcoma cell) whole cell lysate at 20 µg

Lane 5:
Jurkat (human t cell leukemia t lymphocyte from peripheral blood) whole cell lysate at 20 µg

Lane 6:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 7:
HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 70-80 kDa, 36 kDa

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