abcam

ab172730

Rabbit IgG, monoclonal [EPR25A] - Isotype Control

Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) is a rabbit monoclonal antibody used in Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF, ChIP-seq, ChIC/CUT&RUN-seq. - Biophysical QC for unrivalled batch-batch consistency - Over 610 publications

Recombinant | RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Clone number	EPR25A
Purification technique	Affinity purification Protein A
Concentration	1.738 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Application	ChIC/CUT&RUN-seq
Reactivity	Reacts
Dilution info	0.5-2 µg
Notes	-

Application	ChIP-seq
Reactivity	Expected

Dilution info	-
Notes	-

Application	IP
Reactivity	Reacts
Dilution info	-
Notes	-

Application	ICC/IF
Reactivity	Reacts
Dilution info	-
Notes	**Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used.

Application	IHC-P
Reactivity	Reacts
Dilution info	5 µg/mL
Notes	**Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used. Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Application	Flow Cyt
Reactivity	Reacts
Dilution info	-
Notes	**Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used.

Application	WB
Reactivity	Reacts
Dilution info	-
Notes	-

Application	Flow Cyt (Intra)
Reactivity	Reacts
Dilution info	-

Notes	-

Storage

Shipped at conditions	Conditional Ambient
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

Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in ChIC/CUT&RUN-seq, ChIP-seq, Flow Cyt, ICC/IF, IHC-P, IP, and WB.

Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) was first used in a scientific publication in 2014 and has been cited over 617 times in peer reviewed journals. It's performance in Western blot, immunofluorescence, IHC and ChIP in human, mouse and rat samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) is sold in 200 µg selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPR25A - ab210849.

Antibody clone EPR25A is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, HRP, Alexa Fluor® 45, Alexa Fluor® 594, Alexa Fluor® 555, PE, Alexa Fluor® 568, PerCP, FITC, APC, Biotin, Alexa Fluor® 75 (ab19991, ab19993, ab19957, ab2815, ab28568, ab28569, ab29478, ab29613, ab22217, ab223339, ab232814, ab3273, ab32286).

One of the top cited IgG Isotype control antibodies in the market with >1000 citations. IgG Isotype Control antibodies are essential for multiple applications. Isotype Control ab172730 is suitable for ChIC/CUT&RUN-seq, IP, ICC/IF, IHC-P,Flow Cyt, WB. This negative control antibodies help distinguish specific antibody binding from non-specific interactions, ensuring reliable results. Optimize your research with high-quality IgG Isotype Control antibodies for precise data interpretation

Immunogen
Chemical / Small Molecule conjugated to keyhole limpet haemocyanin. KLH is a copper containing oxygen carrier occurring freely dissolved in the hemolymph of many molluscs and arthropods. KLH forms a large complex composed of ~50 kDa subunits.

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 the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant 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

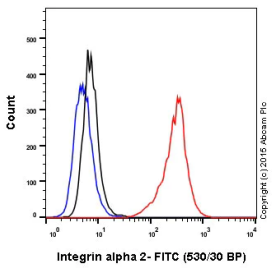
We do not recommend this combination. It is not covered by our product promise.

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.

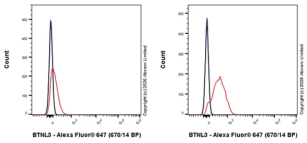
41 product images



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody

Overlay histogram showing A549 (human lung carcinoma) cells stained with ab133557 (red line). The cells were fixed with 2% paraformaldehyde. The cells were then incubated with ab133557 at 1/60 dilution. The secondary antibody used was goat anti-rabbit IgG (FITC) at 1/150 dilution. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730). Unlabelled control (blue line) was cells without incubation with primary antibody and secondary antibody. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 530/30 bandpass filter.
Conjugated versions are available for this clone: Alexa Fluor® 488 (ab199091), Alexa Fluor® 647 (ab199093), R-PE (ab209478), APC (ab232814).



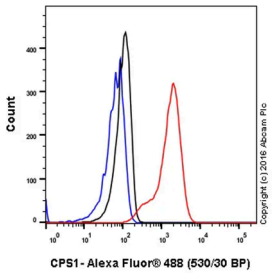
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of SNU-16 (human Stomach epithelial, Right) using rabbit Anti- antibody

Flow cytometric analysis of SNU-16 (human Stomach epithelial, Right) A549 (human lung carcinoma epithelial cell, Left) cells labelling BTNL3 with ab327704 at 1/50 dilution (1ug) / 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® 647, ab150083) at 1/5000 dilution was used as the secondary antibody.

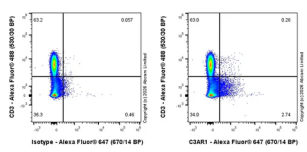
Gated on viable cells. Low expression: A549.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining of HeLa (Human cervix adenocarcinoma epithelial cell) cells using rabbit Anti- antibody

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling CPS1 (red) with ab129076 at a 1/20 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (Black) (ab172730). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).



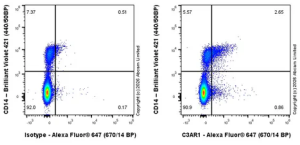
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) culture overnight using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) culture overnight cells labelling with ab327654 at 1/50 dilution (1ug) / 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® 647, ab150083) at 1/5000 dilution was used as the secondary antibody.

Gated on viable cells. Cells were co-stained with CD3 conjugated to Alexa Fluor®488.



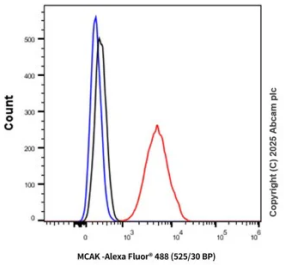
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) culture overnight using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) culture overnight cells labelling with ab327654 at 1/50 dilution (1ug) / 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® 647, ab150083) at 1/5000 dilution was used as the secondary antibody.

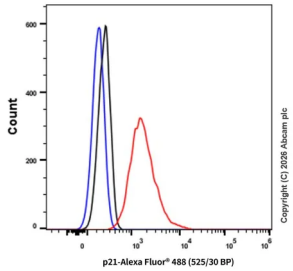
Gated on viable cells. Cells were co-stained with CD14 conjugated to Brilliant Violet 421.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining of HeLa (human cervix adenocarcinoma epithelial cell) cells using rabbit Anti- antibody

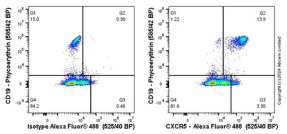
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilised HeLa (human cervix adenocarcinoma epithelial cell) cells labeling MCAK with ab187652 at 1/1000 dilution (Red). Isotype control (ab172730) (black), Unlabelled control (Cells without incubation with primary antibody and secondary antibody) (blue). Secondary antibody - Goat anti-Rabbit IgG H&L (Alexa Fluor® 488, ab150081) preadsorbed used at 1/5000 dilution.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining of HeLa (human cervix adenocarcinoma epithelial cell) using rabbit Anti- antibody

Intracellular flow cytometric analysis of 90% methanol permeabilized HeLa cells labelling p21 with ab109520 at a dilution of 1/100 (red). Cells were fixed with 4% paraformaldehyde. Goat anti-Rabbit IgG H&L (Alexa Fluor® 488, ab150081) preadsorbed (1/5000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



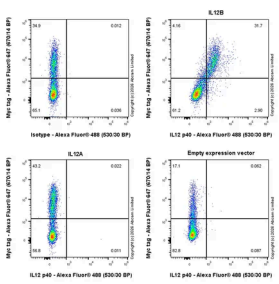
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of human peripheral blood mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab254415 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left). PBMCs were incubated for 30 mins on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab254415 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (1x 10^6 in 100 µl at 0.2 µg/ml (1/11500 dilution)) for 30 mins on ice. The cells were simultaneously stained with CD19.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 dilution for 30 mins on ice

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.

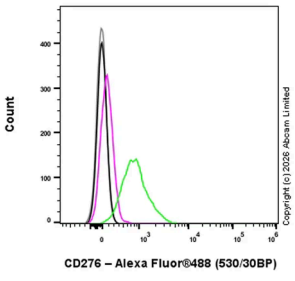


Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized 293T cells transfected with a human IL12B expression vector containing a HA-Myc-tag® (Upper) 293T cells transfected with a human IL12A expression vector containing a HA-Myc-tag® (Lower left) 293T cells transfected with an empty expression vector containing a myc-His-tag® (Lower right) cells labelling IL12 p40 with ab327081 at 1/50000 dilution (0.001ug) / Upper right and Lower compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

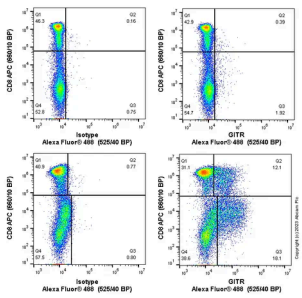
Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric overlay histogram analysis of 4% paraformaldehyde cells labelling CD276 KO HEK293T (ED040783) (Magenta and grey) cells permeabilised with 90% methanol and Parental HEK293T (EDWT04) (Green and black) cells permeabilised with 90% methanol labelling CD276 with ab227679 at 1/20 dilution (Magenta and Green) compared with a Rabbit monoclonal IgG (ab172730) (Black and Grey) - Isotype control. Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150081), at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

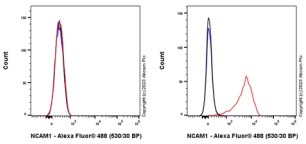
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) (top) or PBMCs treated with anti-CD3 and anti-CD28 antibodies (72 hours) (bottom), with ab237714 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Cells were fixed and permeabilised with BD Cytofix/Cytoperm™ for 20 min. PBMCs were incubated for 30 min at 4°C in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and

non-specific protein-protein interaction followed by the antibody ab237714 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 106 in 100 µl at 0.2 µg/ml (1/10200)) for 30 min at 4°C . The cells were simultaneously stained with CD8.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 4°C

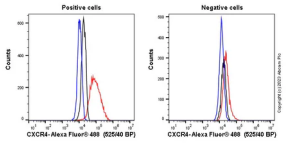
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of MCF7 (human breast adenocarcinoma epithelial cell, Left) - / No-GFP-CD16.NK-92 (CD16 ectopically expressed natural killer(NK-92®) cell with no GFP tag) (Right) cells labelling NCAM1 with ab313779 at 1/500 dilution (0.1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody. Negative control: MCF7 (PMID: 30050775). Gated on viable cells.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

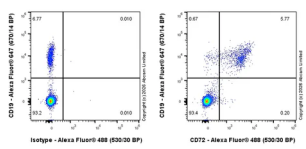
Flow cytometry overlay histogram showing left Jurkat positive cells and right negative HepG2 stained with ab124824 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab124824) (1x 106 in 100µl at 1.0µg/ml (1/1900)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Jurkat Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

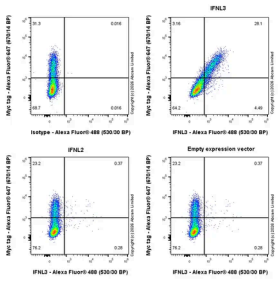
Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling CD72 with ab327352 at 1/500 dilution (0.1ug) / 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. Cells were co-stained with CD19 conjugated to Alexa Fluor®647.

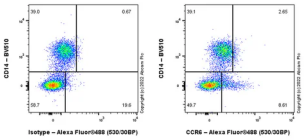


Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized 293T cells transfected with a human IFNL3 expression vector containing a HA-Myc-tag® (Upper) 293T cells transfected with a human IFNL2 expression vector containing a HA-Myc-tag® (Lower left) 293T cells transfected with an empty expression vector containing a myc-His-tag® (Lower right) cells labelling with ab327709 at 1/5000 dilution (0.01ug) / Upper right and Lower right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

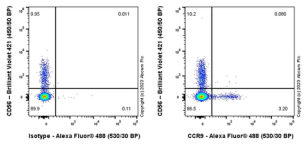
Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human peripheral blood mononuclear cell (PBMC) using rabbit Anti- antibody

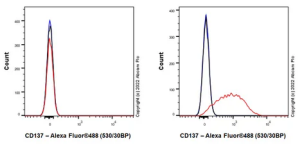
Flow cytometric analysis of / fixed / permeabilized Human peripheral blood mononuclear cell (PBMC) cells labelling CCR6 with ab303672 at 1/500 dilution (0.1ug)/ Right (Red) compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Cells were stained with rabbit IgG or ab303672. Then stained with anti-CD14 conjugated to BV510.Gated on viable cells.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

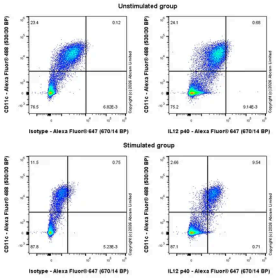
Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling CCR9 with ab313773 at 1/50 dilution (1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody. Cells are co-stained with CD56 conjugated to BV421. Gated on viable cells.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of Jurkat (Human T cell leukemia T lymphocyte, Left) / HDLM-2 (Human Hodgkin lymphoma, Right) cells labelling CD137 with ab300349 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.Negative control: Jurkat (PMID: 25409517).



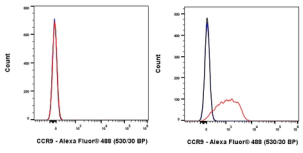
Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 0.1% Tween-20 permeabilized Human PBMC (human peripheral blood mononuclear cell) treated with 100ng/ml GM-CSF and 50ng/ml IL-4 for 6 days then add 10ng/ml TNF alpha(human), 1000U/ml IL-6(human), 10ng/ml IL-1 beta(human) and 1ug/ml PGE2 for 2 days (Lower left and right) Untreated Human PBMC (Upper left and right) cells labelling IL12 p40 with ab327081 at 1/500 dilution (0.1ug) / Upper right and Lower 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® 647, ab150083) at 1/5000 dilution was used as the secondary antibody.

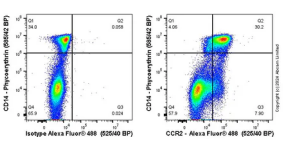
Cells were surface stained with anti-CD11c conjugated to Alexa Fluor®488. Then fixed with 4% PFA for 10 min followed by intracellularly staining with rabbit IgG or our antibody. Maturation of dendritic cells (DCs) is induced by treatment with 100 ng/mL GM-CSF and 50 ng/mL IL-4 for 6 days, followed by the addition of 10 ng/mL TNF-α (human), 1000 U/mL IL-6 (human), 10 ng/mL IL-1β (human), and 1 µg/mL PGE2 for an additional 2 days (PMID: 22882679).



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of Jurkat (human T cell leukemia T lymphocyte from peripheral blood, Left) / MOLT-4 (human lymphoblastic leukemia T lymphoblast, Right) cells labelling CCR9 with ab313773 at 1/50 dilution (1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody. Negative control: Jurkat (PMID: 19525985). Gated on viable cells.



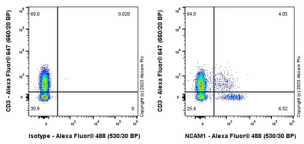
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of human peripheral blood mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab254375 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left). PBMCs were incubated for 30 mins on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab254375 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (1x 10^6 in 100 µl at 0.2 µg/ml (1/11000 dilution)) for 30 mins on ice. The cells were simultaneously stained with CD14.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 dilution for 30 mins on ice

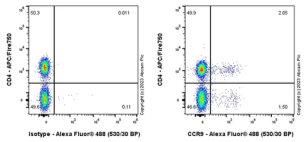
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

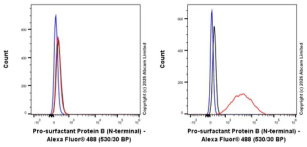
Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling NCAM1 with ab313779 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody. Cells are co-stained with CD3 conjugated to Alexa Fluor®647. Gated on viable cells.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling CCR9 with ab313773 at 1/50 dilution (1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody. Cells are co-stained with CD4 conjugated to APC/Fire750. Gated on viable cells.



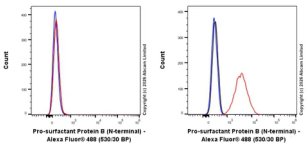
Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining of TT (human thyroid carcinoma epithelial cell) (Right) using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized TT (human thyroid carcinoma epithelial cell) (Right) and BxPC-3 (human pancreas adenocarcinoma epithelial cell) (Left) labelling Pro-surfactant Protein B with ab327883 at 1/500 dilution 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.

Low expression: BxPC-3.



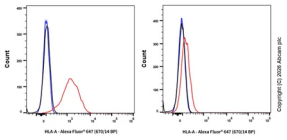
Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining of TT (human thyroid carcinoma epithelial cell) (Right) using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized TT (human thyroid carcinoma epithelial cell) (Right) and BxPC-3 (human pancreas adenocarcinoma epithelial cell) (Left) labelling Pro-surfactant Protein B (Pre-pro and C-terminal pro) with ab327880 at 1/500 dilution 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.

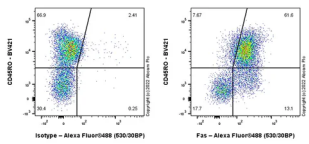
Low expression: BxPC-3.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of A431 (human epidermoid carcinoma epithelial cell) cells using rabbit Anti- antibody

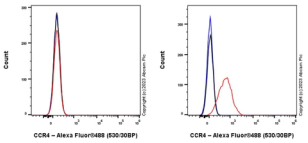
Flow Cytometry analysis of Left: A431 (human epidermoid carcinoma epithelial cell) cells, and Right: MCF7 (human breast adenocarcinoma epithelial cell) cells labeling HLA A with purified ab52922 at 1/200 dilution (0.1µg) (Red). Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabeled control, cells without incubation with primary and secondary antibodies. Gated on viable cells.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human peripheral blood mononuclear cell (PBMC) using rabbit Anti- antibody

Flow cytometric analysis of human peripheral blood mononuclear cell (PBMC) cells labeling Fas with ab307591 at 1/50 dilution (1µg) (Right panel) compared with a Rabbit monoclonal IgG (ab172730) isotype control (Left panel). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells. Cells were stained with rabbit IgG or ab307591, then stained with anti-CD45RO conjugated to BV421.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

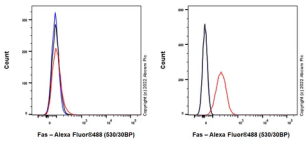
Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of 293T (human embryonic kidney epithelial cell, Left) / HDLM-2 (human Hodgkin lymphoma cell, Right) cells labelling CCR4 with ab315446 at 1/50 dilution (1 ug)/Red

compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

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

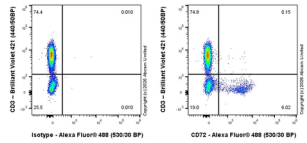
Negative control: 293T. Gated on viable cells.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of K562 (human chronic myelogenous leukemia lymphoblast) (Left panel) / Jurkat (human T cell leukemia T lymphocyte) (Right panel) cells labeling Fas with ab307591 at 1/50 dilution (1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells. Low expression: K-562 (PMID: 9168303).



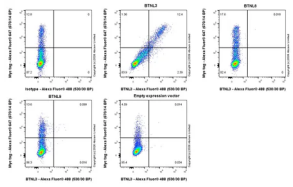
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling CD72 with ab327352 at 1/500 dilution (0.1ug) / 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. Cells were co-stained with CD3 conjugated to Brilliant Violet 421.

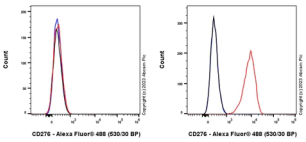


Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

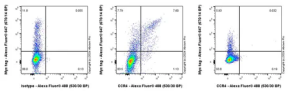
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized 293T cells transfected with a human BTNL3 expression vector containing a HA-Myc-tag® (Upper left and middle) 293T cells transfected with a human BTNL8 expression vector containing a HA-Myc-tag® (Upper right) 293T cells transfected with a human BTNL9 expression vector containing a HA-Myc-tag® (Lower left) 293T cells transfected with an empty expression vector containing a myc-His-tag® (Lower right) cells labelling BTNL3 with ab327704 at 1/5000 dilution (0.01ug) 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.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody



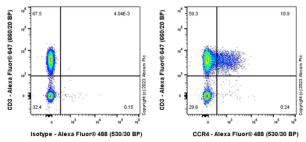
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized Isotype (Left) / 293T cells transfected with a mouse CCR4 expression vector containing a myc-His-tag® (Middle) / 293T cells transfected with an empty expression vector containing a myc-His-tag® (Right) cells labelling CCR4 with ab318958 at 1/5000 dilution (0.01 ug)/Middle and Right (Red) compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

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

Cells were surface stained with isotype control (ab172730) or ab318958. Then fixed with 2% PFA for 10min followed by intracellularly stained with anti-myc tag conjugated to Alexa Fluor® 647.



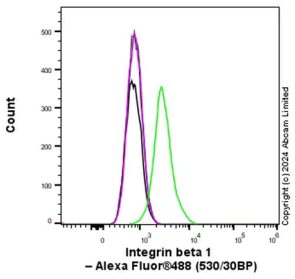
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling CCR4 with ab315446 at 1/50 dilution (1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control.

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

Gated on viable cells. Cells are co-stained with CD3 conjugated to Alexa Fluor®647.

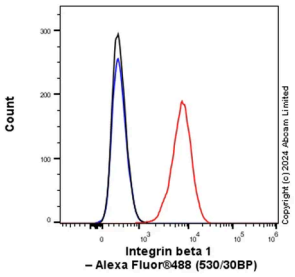


Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Parental HeLa(human cervical adenocarcinoma epithelial cell) (Green)/ITGB1 KO (human Integrin beta-1 knock out) HeLa (Magenta) cells labelling Integrin beta 1 with ab322405 at 1/50 dilution (1ug) / Magenta and Green compared with a Rabbit monoclonal IgG (ab172730) (Black) and Grey 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.

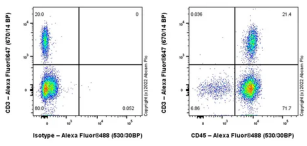


Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining of HepG2(human hepatocellular carcinoma epithelial cell) using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HepG2(human hepatocellular carcinoma epithelial cell) cells labelling Integrin beta 1 with ab322405 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

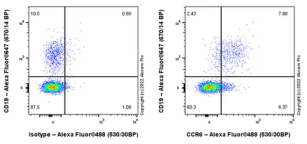
Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human peripheral blood mononuclear cell (PBMC) using rabbit Anti- antibody

Flow cytometric analysis of human peripheral blood mononuclear cells (PBMCs) labelling CD45 with ab303670 at 1/500 dilution (0.1 µg; right) or Rabbit monoclonal IgG isotype control (ab172730; left). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) was used as the secondary antibody at 1/2000 dilution. Cells were then stained with anti-CD3 conjugated to Alexa Fluor® 647. Gated on viable cells.

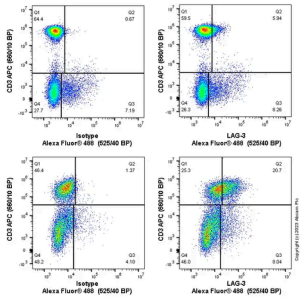


Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human peripheral blood mononuclear cell (PBMC) using rabbit Anti- antibody

Flow cytometric analysis of / fixed / permeabilized Human peripheral blood mononuclear cell (PBMC) cells labelling CCR6 with ab303672 at 1/500 dilution (0.1ug)/ Right (Red) compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without

incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Cells were stained with rabbit IgG or ab303672. Then stained with anti-CD19 conjugated to Alexa Fluor® 647.Gated on viable lymphocytes.



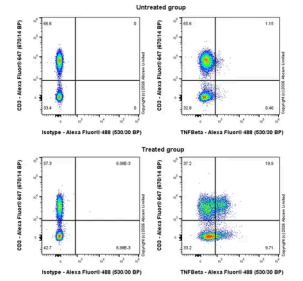
Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) (top) or PBMCs treated with 5 µg/ml phytohaemagglutinin (PHA) for 72 hours (bottom), with ab209236 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). PBMCs were incubated for 30 min on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab209236 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 106 in 100 µl at 5.0 µg/ml (1/402)) for 30min on ice. The cells were simultaneously stained with CD3.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

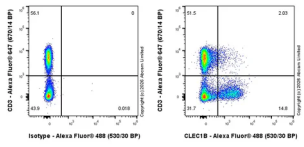
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.



Flow Cytometry (Intracellular) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry scatter plots showing Human PBMC (human peripheral blood mononuclear cell) treated with ab244964, 50ng/ml PMA and 500ng/mL Ionomycin for 24 hours then add 300ng/ml BFA for 20 hours (Lower left and right). Untreated Human PBMC (Upper left and right). The cells were fixed and permeabilised using 4% paraformaldehyde and 0.1% Tween-20. Cells were surface stained with anti-CD3 conjugated to Alexa Fluor®647. Then fixed with 4% PFA for 10 min followed by intracellularly staining with rabbit IgG or our antibody.



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling CLEC1B with ab327705 at 1/500 dilution (0.1ug) / 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.
Cells were co-stained with CD3 conjugated to Alexa Fluor®647.

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