abcam

ab199091

Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control

Anti-Rabbit IgG, monoclonal [EPR25A] - Isotype Control- Alexa Fluor® 488 conjugated (ab199091) is a rabbit recombinant monoclonal antibody used in **Flow Cytometry, ICC/IF**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Conjugation	Alexa Fluor® 488
Excitation/Emission	Ex: 495nm, Em: 519nm
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA
Form	Liquid
Clonality	Monoclonal
Clone number	EPR25A
Purification technique	Affinity purification Protein A
Specificity	Please note Abcam have optimised the validation of this product. In our hands, we observe an increase in background signal intensity with the use of Triton X-100 and would recommend using an alternative permeabilisation method such as methanol or saponin.
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

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, Store in the dark

Notes

Product Specifications
Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt, Flow Cyt (Intra) and ICC/IF.

Quality and Validation
Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) has been cited over 25 times in peer reviewed journals and is trusted by the scientific community.
Flourescent conjugated antibodies are ideal tools for multiplex IHC and flow cytometry experiments.
Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) has 25 independent reviews from customers.
Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) specifically detects rabbit IgG and is sold in 100 µL selling sizes.

Related Products
Conjugation-ready, carrier free format available for antibody clone (EPR25A) - ab210849.

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.

Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@thermofisher.com.

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

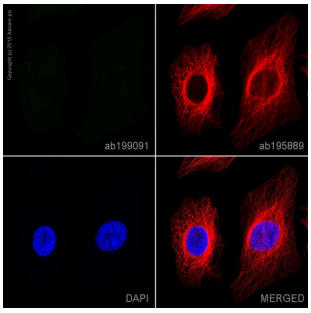
We do not recommend this combination. It is not covered by our product promise.

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

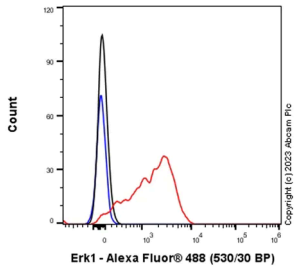


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Immunocytochemistry/ Immunofluorescence staining using rabbit Anti- antibody

Immunofluorescent analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells, fixed with 4% formaldehyde (10 min). The cells were permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab199091 (Rabbit IgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (pseudocolored in red). Nuclear DNA was labeled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

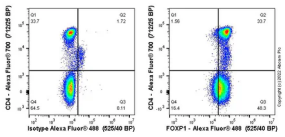
This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry staining of Jurkat (human T cell leukemia T lymphocyte from peripheral blood) using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Jurkat (human T cell leukemia T lymphocyte from peripheral blood) cells labelling ERK1 with ab312910 at 1/50 dilution (1 ug) (Red) compared with a Rabbit monoclonal IgG (ab199091) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).



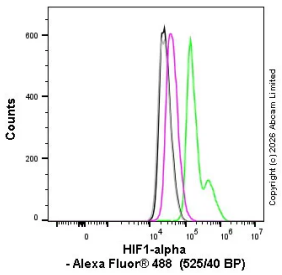
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab303573 (right) or Rabbit IgG (monoclonal) Alexa Fluor®488 (ab199091) isotype (left). Cells were fixed and permeabilized with BD Cytofix/Cytoperm™ for 20 min. 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 (ab303573) or Rabbit IgG (monoclonal) Alexa Fluor®488 (ab199091) isotype (100 µl at 0.2 µg/ml (1/2500)) for 30 min on ice. The cells were simultaneously stained with CD4.

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

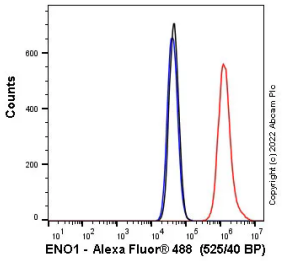
Events were gated on live lymphocytes.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of flow cyt: hct-116 wt using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HCT-116 (green line) and HIF1a knockout HCT-116 stained with ab326123 (magenta line). The cells were fixed with 4% formaldehyde (10 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 (ab326123) (1x 106 in 100µl at 1 µg/ml (1/500)) for 30min at 22°C.Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) in HCT-116 WT cells (black line) and HCT-116-HIF1a KO cells (grey line), used at the same concentration and conditions as the primary antibody. Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

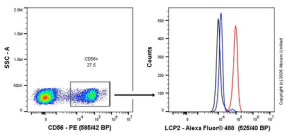
Flow Cytometry (Intracellular) staining of HeLa cells using rabbit Anti- antibody

Flow cytometry overlay histogram showing HeLa cells stained with ab303582 (red line). The cells were fixed with 80 % methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were incubated in 1x PBS containing 10 % normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab303582) (1x 106 in 100µl at 0.2µg/ml (1/2500)) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Alexa Fluor 488 ® (ab199091) used at the same concentration and conditions as the primary antibody. Unlabeled sample (blue line) was also used as a control.

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

This antibody gave a positive signal in HeLa cells fixed with 4% formaldehyde (10 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

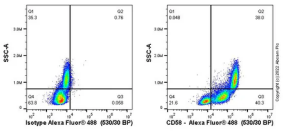
Flow Cytometry (Intracellular) staining of Human PBMC using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 0.1% saponin permeabilized Hu PBMC cells labelling SLP76 with ab328643 at 0.04 ug/mL / Red compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / 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 1/4000 dilution was used as the secondary antibody.

Flow cytometry overlay histogram showing $CD56^+$ human PBMCs (positive cells) stained with ab328643 (red line). Cells were fixed and permeabilised with BD Cytofix/Cytoperm for 20 min. Cells were incubated for 30 min on ice in 1× PBS containing 10 µg/ml human IgG and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions, followed by the antibody ab328643 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1×10^6 cells in 100 µl at 0.04 µg/ml) for 30 min at 22°C. The cells were simultaneously stained with CD56.

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, used at the same concentration and under the same conditions as the primary antibody. An unlabelled sample (without incubation with the primary antibody, blue line) was also used as a control. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor 488), preadsorbed, was incubated at 1/4000 for 30 min at 22°C. Acquisition of >30, 000 events was performed using a 50 mW blue laser (488 nm) and a 525/40 bandpass filter. Events were gated on CD56+ cells.

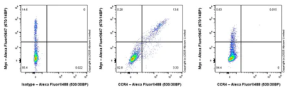


Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab303596 (right) or Rabbit IgG (monoclonal) Alexa Fluor® 488 (ab199091) isotype (left). PBMCs were incubated for 30 min on ice in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab303596) or Rabbit IgG (monoclonal) Alexa Fluor® 488 (ab199091) isotype (1x 106 in 100µl at 0.2 µg/ml (1/2500)) for 30 min on ice.

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

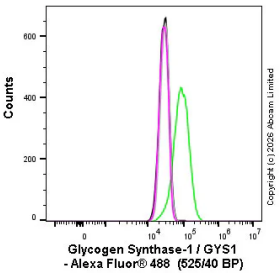


Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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 ab324335 at 1/5000 dilution (0.01ug) / Middle and Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

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

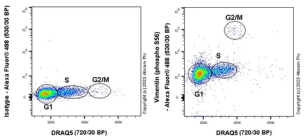


Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of flow cyt: hela wt using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HeLa (green line) and GYS1 knockout HeLa stained with ab326126 (magenta 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 (ab326126) (1x 106 in 100µl at 1 µg/ml (1/500)) for 30min at 22°C.Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) in HeLa WT cells (black line) and HeLa-GYS1 KO cells (grey line), used at the same concentration and conditions as the primary antibody. 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 wild-type HeLa fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15

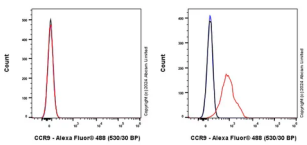
min under the same conditions.This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling Vimentin (phospho S56) with ab312898 at 1/500 dilution (0.1 µg)/Red (Red) compared with a Rabbit monoclonal IgG (ab199091) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Cells were co-stained with DRAQ5 to differentiate cell cycle phase.

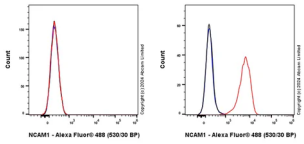


Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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 ab319184 at 1/500 dilution (0.1ug) (Red) compared with a ab199091) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Negative control: Jurkat (PMID: 19525985).
Gated on viable cells.

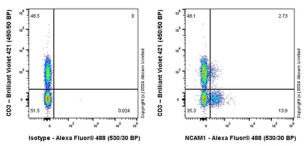


Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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 ab319016 at 1/500 dilution (0.1ug)compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody) (Blue).

Negative control: MCF7 (PMID: 30050775).
Gated on viable cells.



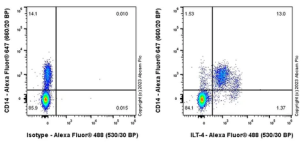
Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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 ab319016 at 1/500 dilution (0.1ug) / Right compared with a Rabbit IgG

monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / Left isotype control and an unlabelled control (cells without incubation with primary antibody) (Blue).

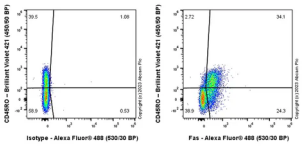
Cells are co-stained with CD3 conjugated to BV421.
Gated on viable cells.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

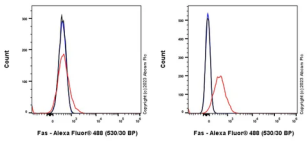
Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling ILT-4 with ab315183 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / Left isotype control. Cells are co-stained with CD14 conjugated to Alexa Fluor® 647. Gated on viable cells.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

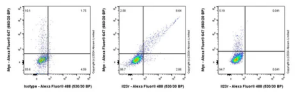
Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) cells labelling Fas with ab314884 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Gated on viable cells. Cells are co-stained with CD45RO conjugated to BV421.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of K-562 (human chronic myelogenous leukemia lymphoblast, Left) / Jurkat (human T cell leukemia T lymphocyte from peripheral blood, Right) cells labelling Fas with ab314884 at 1/500 dilution (0.1 ug)/Red compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Negative control: K-562. Gated on viable cells.

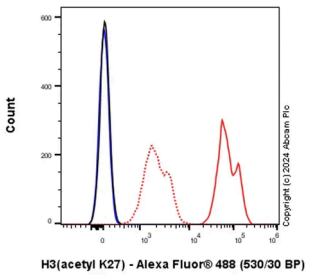


Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized Isotype (Left) / HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with myc-tagged IL-23R expression vector containing a Myc tag (Middle) / 293T cells transfected with an empty vector containing a Myc tag (Right) cells labelling IL-23R with ab319083 at 1/5000 dilution (0.01ug) compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / Left isotype control and an unlabelled control (cells without incubation with primary antibody) (Blue).

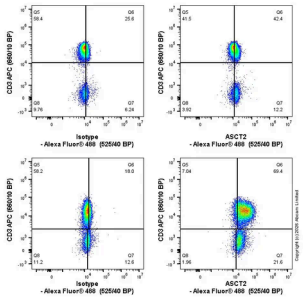
Live cells were surface stained with ab319083 without prior fixation or permeabilization, followed by fixation with 2% PFA and intracellular staining with Myc-tag conjugated to Alexa Fluor® 488.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry staining using rabbit Anti- antibody

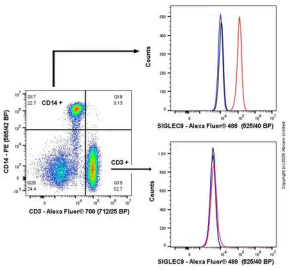
Flow Cytometry analysis of HeLa (human cervical adenocarcinoma epithelial cell) treated with 500ng/mL TSA for 4 hours (Red) / Untreated HeLa (Dotted Red) cells labeling Histone H3 (acetyl K27) with ab245911 at 1/50 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. ab199091 was used as isotype control (black). Unlabelled control/colour: Cells without incubation with primary antibody and secondary antibody (blue).



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of flow cyt: hu pbmc using rabbit Anti- antibody

Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab326135 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated in 1x PBS containing 10 µg/ml human IgG, 10% normal goat serum and BD Pharmingen™ MonoBlock™ Leukocyte Staining Buffer (1/10) to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab326135) (1x 106 in 100µl at 1 µg/ml (1/500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). The cells were simultaneously stained with CD3. Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

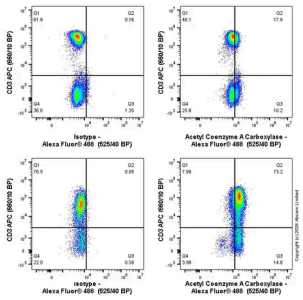
Flow Cytometry staining of Human PBMC using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed / permeabilized Hu PBMC cells labelling SIGLEC9 with ab328645 at 0.04 ug/mL / Red compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / 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 1/4000 dilution was used as the secondary antibody.

Flow cytometry overlay histogram showing top CD14⁺ human PBMCs (positive cells) and bottom CD3⁺ human PBMCs (negative cells) stained with ab328645 (red line). Cells were incubated for 30 min on ice in 1× PBS containing 10 µg/ml human IgG and 10% normal goat serum to block Fc receptors and non☒specific protein-protein interactions, followed by the antibody ab328645 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1×10^6 cells in 100 µl at 0.04 µg/ml) for 30 min on ice. The cells were simultaneously stained with CD14 and CD3. Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, used at the same concentration and under the same conditions as the primary antibody. An unlabelled sample (without incubation with the primary antibody, blue line) was also used as a control. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488), preadsorbed, was

incubated at 1/4000 for 30 min on ice. Acquisition of >30, 000 events was performed using a 50 mW blue laser (488 nm) and a 525/40 bandpass filter. Events were gated on either $CD14^+$ or $CD3^+$ cells.



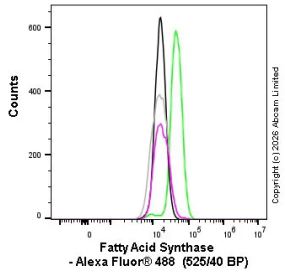
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab326244 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (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 (ab326244) or Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

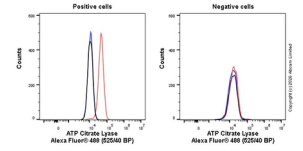
Flow cytometry overlay histogram showing wild-type HAP1 (green line) and FASN knockout HAP1 stained with ab326217 (magenta line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1&# PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab326217) (1x 10^6 in 100µl at 5 µg/ml (1/100)) for 30min at 22°C.

Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control in HAP1 WT cells (black line) and HAP1-FASN KO cells (grey line) was also included.

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 wild-type HAP1 fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of wild-type HAP1 cell line using rabbit Anti- antibody

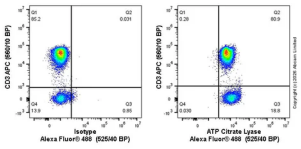
Flow cytometry overlay histogram showing left wild-type HAP1 positive cells and right negative ACLY knockout HAP1 stained with ab205429 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab205429) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) (black line) 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 wild-type HAP1 fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



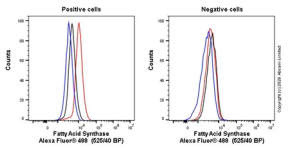
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of Human peripheral mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs) stained with ab205429 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab205429) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) (left). The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of wild-type HAP1 cell line using rabbit Anti- antibody

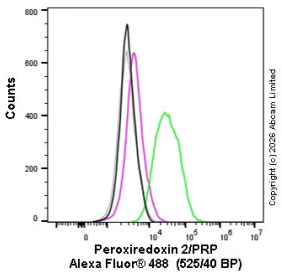
Flow cytometry overlay histogram showing left wild-type HAP1 positive cells and right negative FASN knockout HAP1 stained with ab204660 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab204660) (1x 10^6 in 100µl at 5 µg/ml (1/100)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) (black line) 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 wild-type HAP1 fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of wild-type HAP1 cell line using rabbit Anti- antibody

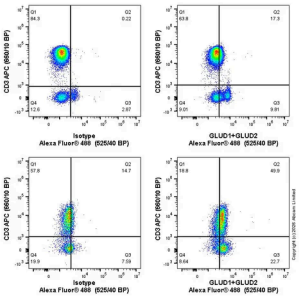
Flow cytometry overlay histogram showing wild-type HAP1 (green line) and PRDX2 knockout HAP1 stained with ab320372 (magenta line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab320372) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) (black line) in HAP1 WT cells (black line) and HAP1-PRDX2 KO cells (grey line), used at the same concentration and conditions as the primary antibody.

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 wild-type HAP1 fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



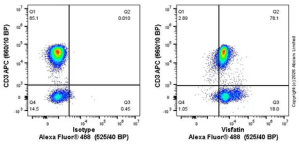
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of Human peripheral mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab204001 (right) or isotype control (left). The cells were fixed using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab204001) (1x 10^6 in 100µl at 5 µg/ml (1/100)) for 30min on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091). The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

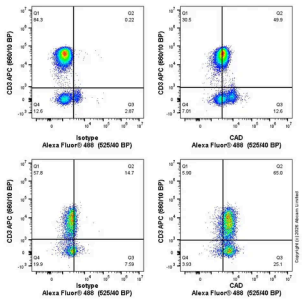
Flow Cytometry (Intracellular) staining of Human peripheral mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs) stained with ab319013 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab319013) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min on ice.

Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) (left). The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



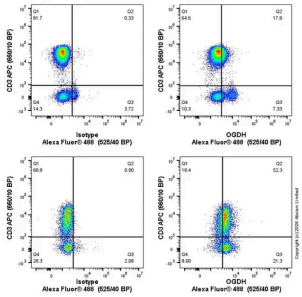
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of Human peripheral mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab309830 (right) or isotype control (left). The cells were fixed using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab309830) (1x 10^6 in 100µl at 5 µg/ml (1/100)) for 30min on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091). The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



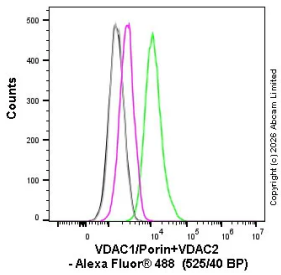
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab326188 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (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 (ab326168) or Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091) (1x 10^6 100µl at 5 µg/ml (1/100)) for 30min on ice. The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

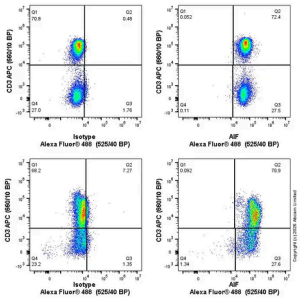
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HAP1 (green line) and VDAC1 knockout HAP1 stained with ab317974 (magenta 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 (ab317974) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) in HAP1 WT cells (black line) and HAP1-VDAC1 KO cells (grey line), used at the same concentration and conditions as the primary antibody.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



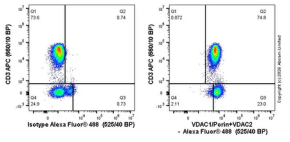
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab309660 (right) or isotype control (left). The cells were fixed using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab309660) (1x 10^6 in 100µl at 1 µg/ml (1/200)) for 30min on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



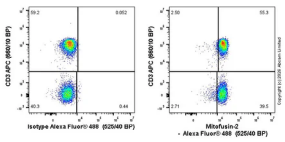
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs), either stained with ab317974 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab317974) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



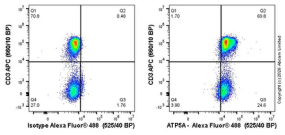
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs), either stained with ab326161 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab326161) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



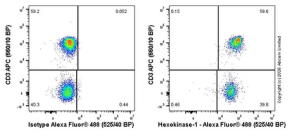
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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

Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs), either stained with ab196467 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab196467) (1x 10^6 in 100µl at 1 µg/ml (1/500)) for 30min on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

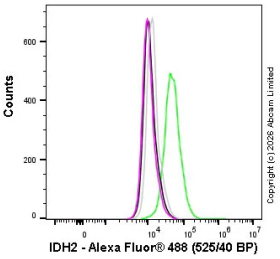
Flow Cytometry (Intracellular) staining of Human peripheral mononuclear cells (PBMCs) using rabbit Anti- antibody

Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs), either stained with ab184818 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated 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 (ab184818) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min

on ice. Isotype control antibody was Recombinant Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

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

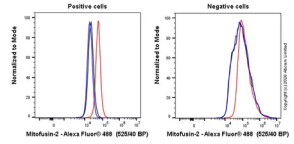
This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of flow cyt: jurkat wt using rabbit Anti- antibody

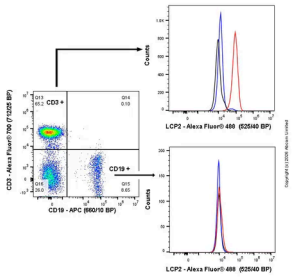
Flow cytometry overlay histogram showing wild-type Jurkat (green line) and IDH2 knockout Jurkat stained with ab326446 (magenta line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab326446) (1x 106 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) in Jurkat WT cells (black line) and Jurkat-IDH2 KO cells (grey line), used at the same concentration and conditions as the primary antibody. 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 wild-type Jurkat fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of flow cyt: hek293t wt using rabbit Anti- antibody

Flow cytometry overlay histogram showing left wild-type HEK293T positive cells and right negative MFN2 knockout HEK293T stained with ab326161 (red line). The cells were fixed with 4% formaldehyde (10 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 (ab326161) (1x 106 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C. Isotype control antibody was Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) 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 data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



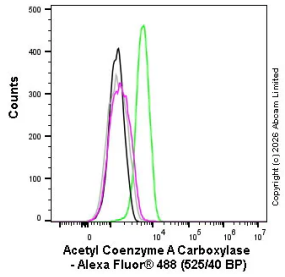
Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry (Intracellular) staining of Human PBMC using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 0.1% saponin permeabilized Hu PBMC cells labelling SLP76 with ab328643 at 0.04 ug/mL / Red compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488) (ab199091) / 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 1/4000 dilution was used as the secondary antibody.

Flow cytometry overlay histogram showing top CD3+ human PBMCs (positive cells) and bottom CD19+ human PBMCs (negative cells) stained with ab328643 (red line). Cells were fixed and permeabilised with BD Cytofix/Cytoperm for 20 min. Cells were incubated for 30 min on ice in 1× PBS containing 10 µg/ml human IgG and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions, followed by the antibody ab328643 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1×10^6 cells in 100 µl at 0.04 µg/ml) for 30 min at 22°C. The cells were simultaneously stained with CD3 and CD19. Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, used at the same concentration and under the same conditions as the primary antibody. An unlabelled sample (without incubation with the primary antibody, blue line) was also used as a control. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor 488), preadsorbed, was incubated at 1/4000 for 30 min at 22°C. Acquisition of >30, 000 events was performed using a 50 mW blue laser (488 nm) and a 525/40 bandpass filter. Events were gated on either CD3+ or CD19+ cells.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

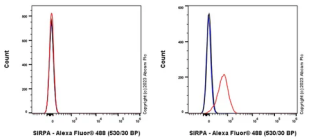
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HAP1 (green line) and ACACA knockout HAP1 stained with ab326244 (magenta 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 (ab326244) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control in HAP1 WT cells (black line) and HAP1-ACACA KO cells (grey line) was also included.

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

This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Flow Cytometry staining of THP-1 (human monocytic leukemia monocyte, Right) using rabbit Anti- antibody

Flow cytometric analysis of permeabilized Jurkat (human T cell leukemia T lymphocyte from peripheral blood, Left). THP-1 (human monocytic leukemia monocyte, Right) cells labelling SIRP alpha with ab312902 at 1/500 dilution (0.1 ug)/Red (Red) compared with a Rabbit monoclonal IgG (ab199091) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Negative control: Jurkat.

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