abcam

ab199093

Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control

Anti-Rabbit IgG, monoclonal [EPR25A] - Isotype Control- Alexa Fluor® 647 conjugated (ab199093) is a rabbit recombinant monoclonal antibody used in **Flow Cytometry, ICC/IF**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 10 publications

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Conjugation	Alexa Fluor® 647
Excitation/Emission	Ex: 650nm, Em: 665nm
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® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt and ICC/IF .

Quality and Validation
Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) has been cited over 19 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® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) has 19 independent reviews from customers.
Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) 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

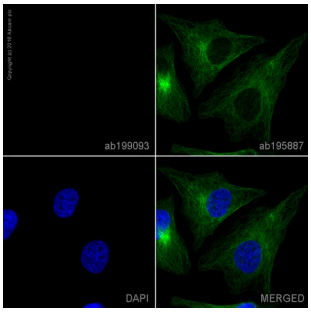
We do not recommend this combination. It is not covered by our product promise.

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

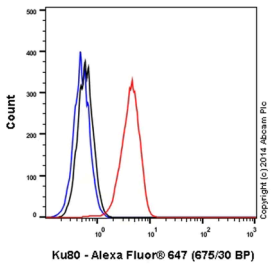


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab199093 (Rabbit IgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). 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).

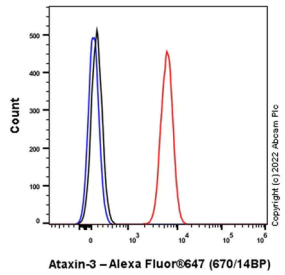


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry staining using rabbit Anti- antibody

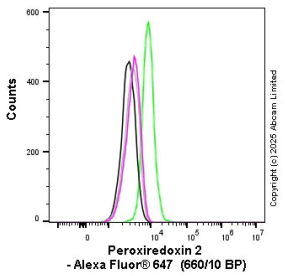
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab198587 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab198587, 1/50 dilution) for 30 min at 22°C.
Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 647 (ab199093) used at the same concentration and conditions as the primary antibody. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a solid-state 25mW red diode laser (635 nm) and 675/30 bandpass filter.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized hela(human cervical adenocarcinoma epithelial cell) cells labelling Ataxin 3 with ab315423 at 1/50 dilution (1 ug)/Red compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

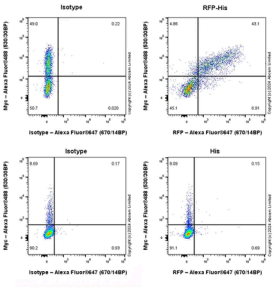
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HAP1 (green line) and PRDX2 knockout HAP1 stained with ab197041 (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 (ab197041) (1x 10^6 in 100µl at 0.008 µg/ml (1/62500 dilution)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control 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 40 mW Red laser (638nm) and 660/10 bandpass filter.

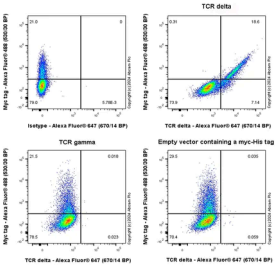


Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized 293T cells transfected with a Red fluorescent protein expression vector containing a His-tag (upper) / 293T cells transfected with an empty vector containing a His tag (low) cells labelling RFP with ab323758 at 1/500 dilution (0.1ug) / Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

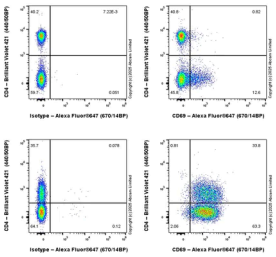
Cells are co-stained with Myc tag conjugated to Alexa Fluor®488.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Isotype / 293T (human embryonic kidney epithelial cell) transfected with a TRDC (human TCR delta) protein expression vector containing a myc-His tag(Up Right) / 293T transfected with a TRGC1 protein (human TCR gamma) expression vector(Down Left) / 293T transfected with an empty vector containing a myc-His tag(Down Right) cells labelling TCR delta with ab317716 at 1/500 dilution (0.1ug)/ Up Right, Down Left and Down Right (Red) compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

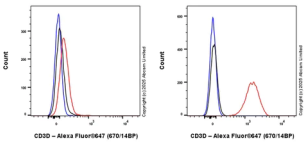


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry staining of Human PBMC (human peripheral blood mononuclear cell) treated with 500ng/ml ionomycin and 10ng/ml PMA for 3 hours (Lower left and right) / Untreated control (Upper left and right) using rabbit Anti- antibody

Flow cytometric analysis of Human PBMC (human peripheral blood mononuclear cell) treated with 500ng/ml ionomycin and 10ng/ml PMA for 3 hours (Lower left and right) / Untreated control (Upper left and right) cells labelling CD69 with ab325108 at 1/500 dilution (0.1ug) / Upper right and Lower right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control.

Cells were stained with anti-CD4 conjugated to Brilliant Violet 421.
Gated on viable lymphoid Cells.

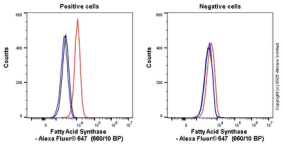


Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (human monocytic leukemia monocyte, Left) and Jurkat (human T cell leukemia T lymphocyte from peripheral blood, Right) cells labelling CD3D with ab198937 at 1/5000 dilution (0.01ug) / Red compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Negative control: THP-1.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

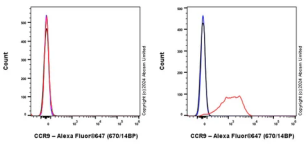
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing left wild-type HAP1 positive cells and right negative FASN knockout HAP1 stained with ab319690 (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 (ab319690) (1x 10^6 in 100µl at 0.008 µg/ml (1/62500 dilution)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 647 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 40 mW Red laser (638nm) and 660/10 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.

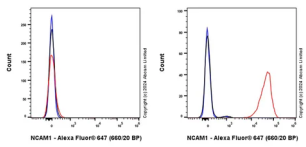


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab320780 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) (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.

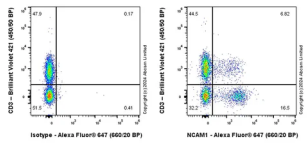


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab319017 at 1/500 dilution (0.1ug)compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody) (Blue).

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

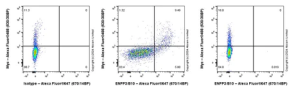


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab319017 at 1/500 dilution (0.1ug) / Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / 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.



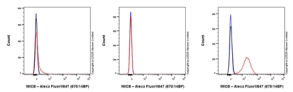
Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry staining using rabbit Anti- antibody

Flow cytometric analysis of Isotype (Left) / HEK-293T (human embryonic kidney epithelial cell) transfected with Myc-tagged ENPP3 overexpression construct (Middle) / 293T transfected with an empty vector containing a myc-His tag (Right) cells labelling ENPP3/B10 with ab319126 at 1/500 dilution (0.1ug) / Middle and Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype

Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody) (Blue).

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

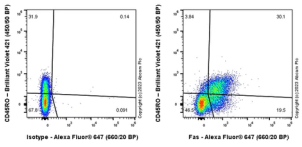


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry staining using rabbit Anti- antibody

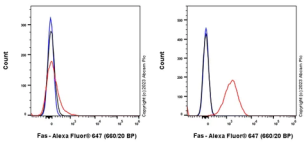
Flow cytometric analysis of 293T (human embryonic kidney epithelial cell, Left) / Daudi (human Burkitt's lymphoma lymphoblast, Middle) / K-562 (human chronic myelogenous leukemia lymphoblast, Right) cells labelling MICB with ab325223 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Gated on viable cells.
Negative control: 293T, Daudi (PMID: 28154561).



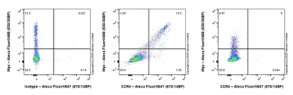
Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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



Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry staining using rabbit Anti- antibody

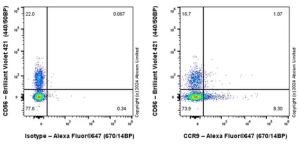


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab324334 at 1/5000 dilution (0.01ug) / Middle and Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / 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® 488.

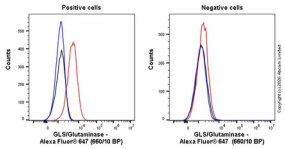


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab320780 at 1/500 dilution (0.1ug) / Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Cells are co-stained with CD56 conjugated to Brilliant Violet 421.
Gated on viable cells.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

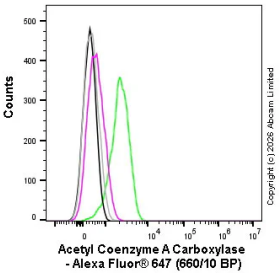
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing left wild-type HAP1 positive cells and right negative GLS knockout HAP1 stained with ab326270 (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 (ab326270) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 647 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 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

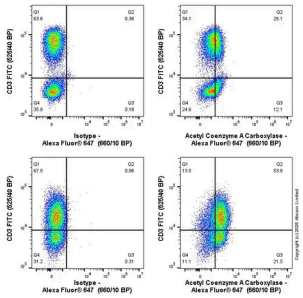
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HAP1 (green line) and ACACA knockout HAP1 stained with ab326245 (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 (ab326245) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Alexa Fluor® 647 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 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



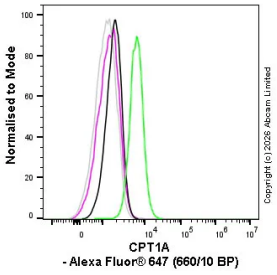
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab326245 (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 (ab326245) or Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6 100µl at 0.04 µg/ml (1/12500)) for 30min on ice. The cells were simultaneously stained with CD3.

Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

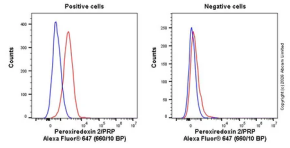
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HAP1 (green line) and CPT1A knockout HAP1 stained with ab326250 (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 (ab326250) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min at 22°C.

Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control in HAP1 WT cells (black line) and HAP1-CPT1A KO cells (grey line) was also included.

Acquisition of >5000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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

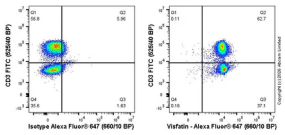
Flow cytometry overlay histogram showing left wild-type HAP1 positive cells and right negative PRDX2 knockout HAP1 stained with ab320373 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab320373) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) (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 40 mW Red laser (638nm) and 660/10 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.



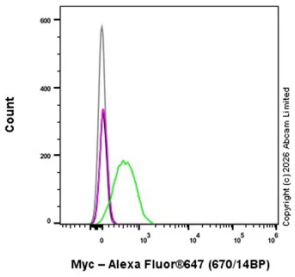
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab318236 (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 (ab318236) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) (left). The cells were simultaneously stained with CD3.

Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

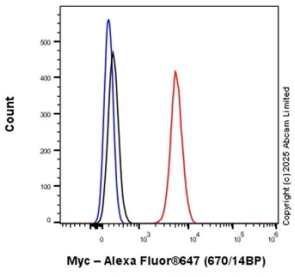
This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

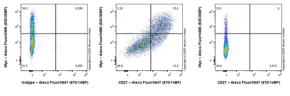
Flow cytometric overlay histogram of 4% paraformaldehyde-fixed, 90% methanol permeabilised parental HEK293T (human embryonic kidney epithelial cell) (EDWT04) cells (green and black histograms) and MYC KO HEK293T (MYC knockout HEK293T) cells (magenta and grey histograms) labelling c-Myc with ab190560 at 1/5000 dilution (0.01 µg) (green and magenta histograms). Isotype control antibody (black and grey histograms) was Rabbit IgG monoclonal [EPR25A] - Isotype Control Alexa Fluor® 647 (ab199093) used at the same concentration and conditions as the primary antibody.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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

Flow cytometric overlay histogram of 4% paraformaldehyde-fixed, 90% methanol permeabilised HeLa (human cervical adenocarcinoma epithelial cell) cells labelling c-Myc with ab190560 at 1/50 dilution (1 µg) (red line). Isotype control antibody (black line) was Rabbit IgG monoclonal [EPR25A] - Isotype Control Alexa Fluor® 647 (ab199093) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

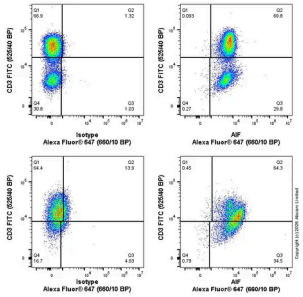


Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Isotype (Left) / 293T cells transfected with a mouse CD27 expression vector (Middle) / 293T cells transfected with an empty expression vector (Right) cells labelling CD27 with ab324178 at 1/5000 dilution (0.01ug) / Middle and Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control.

Cells were co-stained with Myc tag conjugated to Alexa Fluor®488.



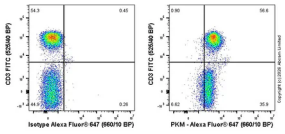
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab196847 (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 (ab196847) (1x 10^6 in 100µl at 0.008 µg/ml (1/62500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



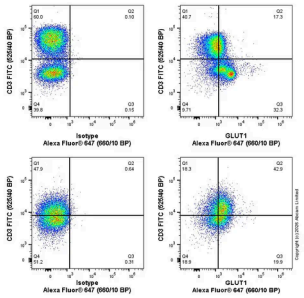
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab214257 (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 (ab214257) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



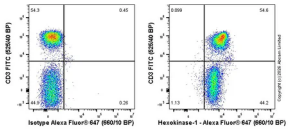
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab195020 (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 (ab195020) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

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



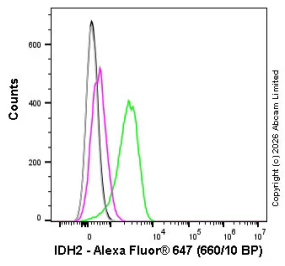
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab197864 (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 (ab197864) (1x 10^6 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3.

Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.

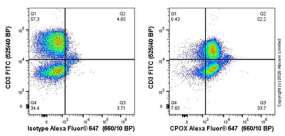
This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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

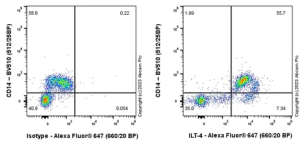
Flow cytometry overlay histogram showing wild-type Jurkat (green line) and IDH2 knockout Jurkat stained with ab326447 (magenta line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab326447) (1x 106 in 100µl at 0.0016 µg/ml (1/312500)) for 30min at 22°C.Isotype control antibody was Alexa Fluor® 647 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 data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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

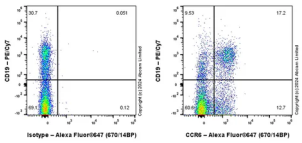
Flow cytometry scatter plots showing human peripheral mononuclear cells (PBMCs), either stained with ab326179 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells were incubated in 1x PBS containing 10 µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (ab326179) (1x 106 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control. The cells were simultaneously stained with CD3. Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab313898 at 1/500 dilution (0.1 ug)/Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Cells are co-stained with CD14 conjugated to BV510. Gated on viable cells.

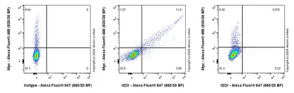


Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 labelling CCR6 with ab322226 at 1/500 dilution (0.1ug) / Right compared with a Rabbit IgG monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Cells were co-stained with anti-CD19 conjugated to PE-Cy7.
Gated on viable cells.



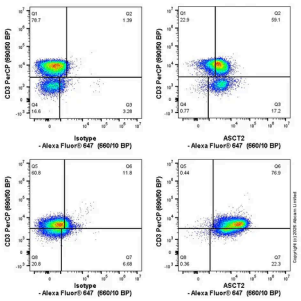
Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab319084 at 1/5000 dilution (0.01ug) compared with a Rabbit IgG monoclonal [EPR25A] - Isotype

Control (Alexa Fluor® 647) (ab199093) / Left isotype control and an unlabelled control (cells without incubation with primary antibody) (Blue).

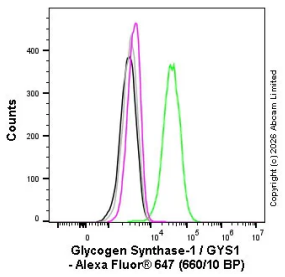
Live cells were surface stained with ab319084 without prior fixation or permeabilization, followed by fixation with 2% PFA and intracellular staining with Myc-tag conjugated to Alexa Fluor® 488.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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

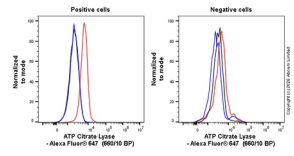
Flow cytometry scatter plots showing untreated human peripheral mononuclear cells (PBMCs) (top) and PBMCs activated with 5 µg/ml PHA for 18h (bottom), either stained with ab326136 (right) or isotype control (left). The cells were fixed and permeabilised using BD CytoFix/CytoPerm™ (20 min). The cells 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 (ab326136) (1x 106 in 100µl at 0.2 µg/ml (1/2500)) for 30min on ice. Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). The cells were simultaneously stained with CD3. Acquisition of >30000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.This data was acquired using a Beckman Coulter CytoFlexLX flow cytometer.



Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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 ab326127 (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 (ab326127) (1x 106 in 100µl at 0.008 µg/ml (1/62500)) for 30min at 22°C.Isotype control antibody was Alexa Fluor® 647 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 40 mW Red laser (638nm) and 660/10 bandpass filter.This antibody gave a positive signal in wild-type HeLa 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® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

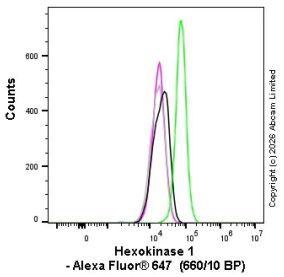
Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing left wild-type HAP1 positive cells and right negative ACLY knockout HAP1 stained with ab205430 (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 (ab205430) (1x 10^6 in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) used at the same concentration and conditions as the primary antibody.

Acquisition of >5000 events were collected using a 40 mW Red laser (638nm) and 660/10 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.



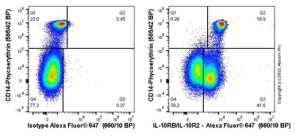
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Flow Cytometry (Intracellular) staining using rabbit Anti- antibody

Flow cytometry overlay histogram showing wild-type HEK293T (green line) and HK1 knockout HEK293T stained with ab197864 (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 (ab197864) (1x 10^6 in 100µl at 5 µg/ml (1/100)) for 30min at 22°C.

Isotype control antibody was Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) in HEK293T WT cells (black line) and HEK293T-HK1 KO cells (grey line), used at the same concentration and conditions as the primary antibody.

Acquisition of >5000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.



Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

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

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