abcam

Product datasheet

Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free ab256355

KO VALIDATED Recombinant RabMAb

5 Images

Overview

Product name	Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free
Description	Rabbit monoclonal [EPR22565-204] to IL-6 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, Flow Cyt (Intra) **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HUVEC treated with LPS then BFA (Brefeldin A) whole cell lysate. ICC/IF: HUVEC treated with LPS then BFA (Brefeldin A) cells. Flow Cyt (intra): HUVEC treated with LPS then BFA (Brefeldin A) cells. IP: HUVEC treated with LPS then BFA (Brefeldin A) whole cell lysate.
General notes	ab256355 is the carrier-free version of **ab233551**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22565-204
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256355 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 21, 28 kDa (predicted molecular weight: 24 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes Is unsuitable for IHC-P.

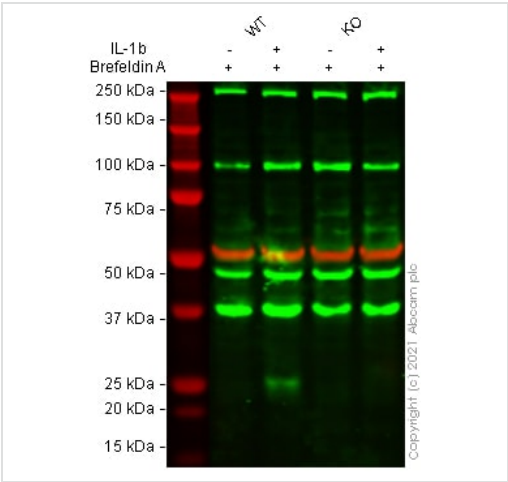
Target

Function Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation Acts on B-cells, T-cells, hepatocytes, hematopoeitic progenitor cells and cells of the CNS. Also acts as a myokine. It is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance.

Involvement in disease Genetic variations in IL6 are associated with susceptibility to rheumatoid arthritis systemic juvenile (RASJ) [MIM:604302]. An inflammatory articular disorder with systemic-onset beginning before the age of 16. It represents a subgroup of juvenile arthritis associated with severe extraarticular features and occasionally fatal complications. During active phases of the disorder, patients display a typical daily spiking fever, an evanescent macular rash, lymphadenopathy,

	hepatosplenomegaly, serositis, myalgia and arthritis. Note=A IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in HIV-infected men.
Sequence similarities	Belongs to the IL-6 superfamily.
Post-translational modifications	N- and O-glycosylated.
Cellular localization	Secreted.

Images



Western blot - Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free (ab256355)

All lanes : Anti-IL-6 antibody [EPR22565-204] (**ab233551**) at 1/1000 dilution

Lane 1 : Wild-type A549 Vehicle Control IL-1b (0 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate
Lane 2 : Wild-type A549 Treated IL-1b (20 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate
Lane 3 : IL-6 knockout A549 Vehicle Control IL-1b (0 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate
Lane 4 : IL-6 knockout A549 Treated IL-1b (20 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate

Lysates/proteins at 30 µg per lane.

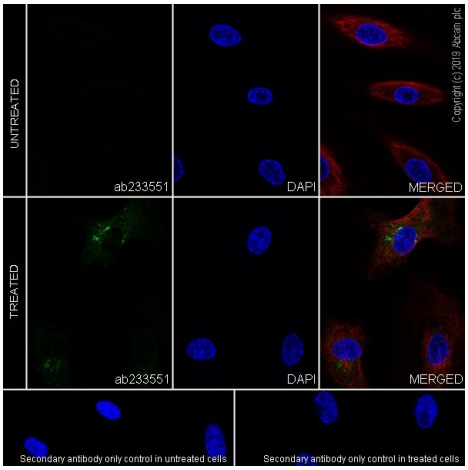
Performed under reducing conditions.

Predicted band size: 24 kDa
Observed band size: 25 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab233551**).

False colour image of Western blot: Anti-IL-6 antibody [EPR22565-204] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab233551** was shown to bind specifically to IL-6. A band was observed at 25 kDa in wild-type A549 cell lysates with no signal observed at this size in IL6 knockout cell line **ab273751** (knockout cell lysate **ab275501**). To generate this image, wild-type and IL6 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then

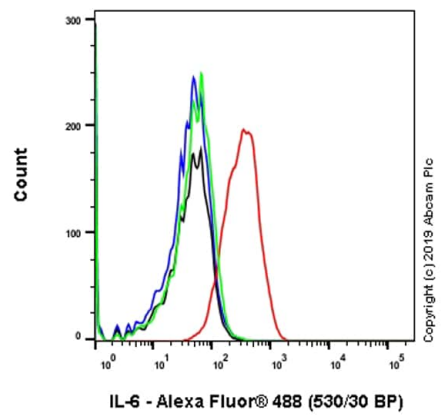
transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free (ab256355)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HUVEC (human umbilical vein endothelial cell) cells labeling IL-6 with **ab233551** at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in HUVEC cells treated with lipopolysaccharide (0.5µg/ml) for 4 h, then together with Brefeldin A (300ng/ml) for another 20h. The nuclear counterstain is DAPI (blue). Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red). The negative control is the secondary antibody only.

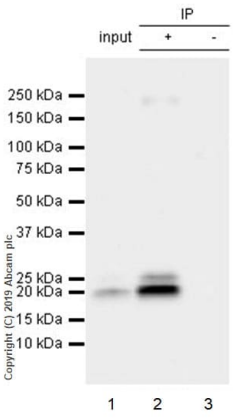
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab233551**).



Flow Cytometry (Intracellular) - Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free (ab256355)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HUVEC (Human umbilical vein endothelial cell) treated with 0.5ug/ml LPS for 4h, then together with 300ng/ml BFA for another 20h (Red) / Untreated control (Green), labeling IL-6 with **ab233551** at 1/400 (red) compared with a Rabbit monoclonal IgG (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**), at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab233551**).



Immunoprecipitation - Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free (ab256355)

IL-6 was immunoprecipitated from 0.35 mg HUVEC (Human umbilical vein endothelial cell) treated with 0.5µg/ml LPS for 4h, then together with 300ng/ml BFA for another 20h whole cell lysate with **ab233551** at 1/20 dilution. Western blot was performed from the immunoprecipitate using **ab233551** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used at 1/5000 dilution.

Lane 1: HUVEC treated as above whole cell lysate 10 µg (Input).

Lane 2: **ab233551** IP in HUVEC treated as above whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab233551** in HUVEC treated as above whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: 15 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab233551**).

Anti-IL-6 antibody [EPR22565-204] - BSA and Azide free (ab256355)

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