abcam

Product datasheet

Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free ab219728

Recombinant RabMAb

22 References 12 Images

Overview

Product name Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free

Description Rabbit monoclonal [E132] to JAK2 (phospho Y1007 + Y1008) - BSA and Azide free

Host species Rabbit

Specificity This antibody is phospho-specific and only detects phosphorylated JAK2 on Tyrosine 1007 and

1008 (pY1007+Y1008). According to our ELISA results, this antibody preferentially recognizes phospho Y1007. Stimulation may be required to allow detection of the phosphorylated protein. Please see images below for recommended treatment conditions and positive controls.

The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), WB, IHC-P, Dot blot, ELISA

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Hepa1-6, MEF, C6, Jurkat treated with Pervanadate and Jurkat cell lysates. IHC-P: Human

differentiated squamous cell carcinoma tissue. ICC/IF: Jurkat cells (treated with Pervanadate). Flow Cyt (intra): Jurkat starved of serum for 16 hours then treated with 1mM Pervanadate for 30

minutes.

General notes ab219728 is the carrier-free version of <u>ab32101</u>.

Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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.

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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: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number E132
Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab219728 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
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 130 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Dot blot		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.

Target

Function

Non-receptor tyrosine kinase involved in various processes such as cell cycle progression, apoptosis, mitotic recombination, genetic instability and histone modifications. In the cytoplasm, plays a pivotal role in signal transduction via its association with cytokine receptors, which constitutes an initiating step in signaling for many members of the cytokine receptor superfamily including the receptors for growth hormone (GHR), prolactin (PRLR), leptin (LEPR), erythropoietin (EPOR), granulocyte-macrophage colony-stimulating factor (CSF2), thrombopoietin (THPO) and multiple interleukins. Following stimulation with erythropoietin (EPO) during erythropoiesis, it is autophosphorylated and activated, leading to its association with erythropoietin receptor (EPOR) and tyrosine phosphorylation of residues in the EPOR cytoplasmic domain. Also involved in promoting the localization of EPOR to the plasma membrane. Also acts downstream of some G-protein coupled receptors. Plays a role in the control of body weight (By similarity). Mediates angiotensin-2-induced ARHGEF1 phosphorylation. In the nucleus, plays a key role in chromatin by specifically mediating phosphorylation of 'Tyr-41' of histone H3 (H3Y41ph), a specific tag that promotes exclusion of CBX5 (HP1 alpha) from chromatin.

Tissue specificity

Involvement in disease

Expressed in blood, bone marrow and lymph node.

Note=Chromosomal aberrations involving JAK2 are found in both chronic and acute forms of eosinophilic, lymphoblastic and myeloid leukemia. Translocation t(8;9)(p22;p24) with PCM1 links the protein kinase domain of JAK2 to the major portion of PCM1. Translocation t(9;12)(p24;p13) with ETV6.

Defects in JAK2 are a cause of susceptibility to Budd-Chiari syndrome (BCS) [MIM:600880]. It is a syndrome caused by obstruction of hepatic venous outflow involving either the hepatic veins or the terminal segment of the inferior vena cava. Obstructions are generally caused by thrombosis and lead to hepatic congestion and ischemic necrosis. Clinical manifestations observed in the majority of patients include hepatomegaly, right upper quadrant pain and abdominal ascites. Budd-Chiari syndrome is associated with a combination of disease states including primary myeloproliferative syndromes and thrombophilia due to factor V Leiden, protein C deficiency and antithrombin III deficiency. Budd-Chiari syndrome is a rare but typical complication in patients with polycythemia vera.

Defects in JAK2 are a cause of polycythemia vera (PV) [MIM:263300]. A myeloproliferative disorder characterized by abnormal proliferation of all hematopoietic bone marrow elements, erythroid hyperplasia, an absolute increase in total blood volume, but also by myeloid leukocytosis, thrombocytosis and splenomegaly.

Defects in JAK2 gene may be a cause of essential thrombocythemia (ET) [MIM:187950]. ET is characterized by elevated platelet levels due to sustained proliferation of megakaryocytes, and frequently lead to thrombotic and haemorrhagic complications.

Defects in JAK2 are a cause of myelofibrosis (MYELOF) [MIM:254450]. Myelofibrosis is a disorder characterized by replacement of the bone marrow by fibrous tissue, occurring in association with a myeloproliferative disorder. Clinical manifestations may include anemia, pallor, splenomegaly, hypermetabolic state, petechiae, ecchymosis, bleeding, lymphadenopathy, hepatomegaly, portal hypertension.

Defects in JAK2 are a cause of acute myelogenous leukemia (AML) [MIM:601626]. AML is a malignant disease in which hematopoietic precursors are arrested in an early stage of development.

Sequence similarities

Belongs to the protein kinase superfamily. Tyr protein kinase family. JAK subfamily.

Contains 1 FERM domain.

Contains 1 protein kinase domain.

Contains 1 SH2 domain.

Domain

Possesses 2 protein kinase domains. The second one probably contains the catalytic domain, while the presence of slight differences suggest a different role for protein kinase 1.

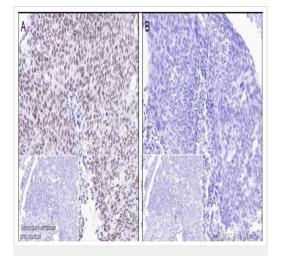
Post-translational modifications

Autophosphorylated, leading to regulate its activity. Leptin promotes phosphorylation on tyrosine residues, including phosphorylation on Tyr-813. Autophosphorylation on Tyr-119 in response to EPO down-regulates its kinase activity. Autophosphorylation on Tyr-868, Tyr-966 and Tyr-972 in response to growth hormone (GH) are required for maximal kinase activity.

Cellular localization

Endomembrane system. Nucleus.

Images

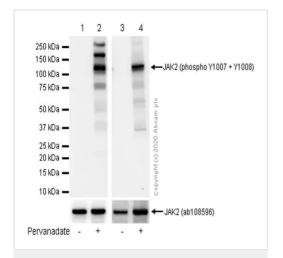


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

ab32101 showing positive staining in Human differentiated squamous cell carcinoma of the cervix tissue at 1/10000 dilution. Goat Anti-Rabbit lgG H&L (HRP) was used as secondary antibody. Antigen retreival was carried out by Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

Nuclear staining on human differentiated squamous cell carcinoma of the cervix without alkaline phosphatase treatment (image A). No staining on human differentiated squamous cell carcinoma of the cervix with alkaline phosphatase treatment (image B)



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

All lanes: Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) at 1/1000 dilution

Lane 1: Hepa1-6 (Mouse hepatoma epithelial cell) whole cell lysate

Lane 2: Hepa1-6 (Mouse hepatoma epithelial cell) treated with 100 µM pervanadate for 30 minutes whole cell lysate

Lane 3 : MEF (Mouse embryonic fibroblast (immortalized)) whole cell lysate

Lane 4 : MEF (Mouse embryonic fibroblast (immortalized)) treated with 100 µM pervanadate for 30 minutes whole cell lysate

Lysates/proteins at 15 µg per lane.

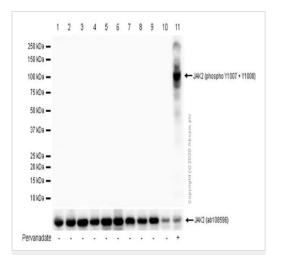
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 130 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32101</u>).

The extra bands are undefined.



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

All lanes : Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101)

Lane 1: Mouse hippocampus lysate

Lane 2: Mouse P240 hippocampus lysate

Lane 3: Mouse P7 hippocampus lysate

Lane 4: Rat hippocampus lysate

Lane 5: Rat P7 hippocampus lysate

Lane 6: Rat brain cortex lysate

Lane 7: Human brain lysate

Lane 8: Mouse brain lysate

Lane 9: Rat brain lysate

Lane 10 : C6 (Rat glial tumor glial cell) whole cell lysateLane 11 : C6 (Rat glial tumor glial cell) treated with 50mM

pervanadate for 5 minutes whole cell lysate

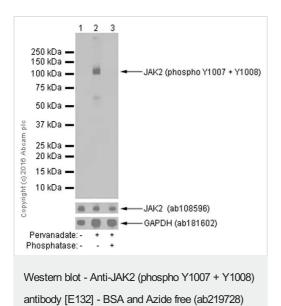
Lysates/proteins at 15 µg per lane.

Secondary

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

Predicted band size: 130 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).



All lanes : Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) at 1/5000 dilution

Lane 1: Untreated Jurkat cells whole cell lysates

Lane 2: Jurkat cells were treated with 50mM Pervanadate for 5 minutes whole cell lysates

Lane 3 : Jurkat cells were treated with 50mM Pervanadate for 5 minutes whole cell lysates. Then the membrane was incubated with Alkaline phosphatase

Lysates/proteins at 10 µg per lane.

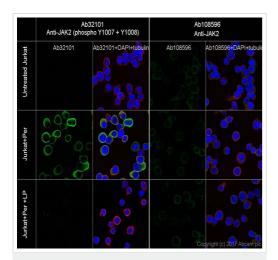
Secondary

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

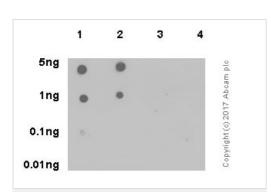
Predicted band size: 130 kDa Observed band size: 120 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

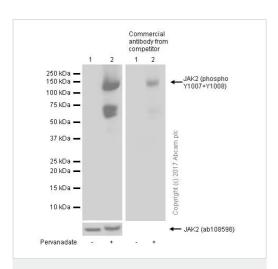
Blocking and diluting buffer 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] -BSA and Azide free (ab219728)



Dot Blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

Immunocytochemistry/Immunofluorescence analysis of Jurkat +/pervanadate (1mM, 30min) and Jurkat + pervanadate (1mM,
30min) + LP cells labelling JAK2 (phospho Y1007 + Y1008) with
ab32101 at a dilution of 1/1000. Cells were fixed with 4%
paraformaldehyde and permeabilized with 0.1% tritonX-100.
ab150077 (goat anti-rabbit lgG Alexa Fluor® 488) (1/1000) was
used as the secondary antibody. The cells were co-stained with
ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule
Marker (Alexa Fluor® 594)) at a 1/200 dilution. Nuclei
counterstained with DAPI (blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

Dot blot analysis of JAK2 (phospho Y1007 & Y1008) phospho peptide (Lane 1), JAK2 (phospho Y1007) phospho peptide (Lane 2), JAK2 (phospho Y1008) phospho peptide (Lane 3) and JAK2 non-phospho peptide (Lane 4) labelling JAK2 (phospho Y1007 & Y1008) with ab32101 at a dilution of 1/1000. A Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) was used as the secondary antibody at a dilution of 1/20,000. Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

All lanes : Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) at 1/1000 dilution

Lane 1: Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates

Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) treated with 50mM Pervanadate for 5 minutes whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

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

Predicted band size: 130 kDa **Observed band size:** 120 kDa

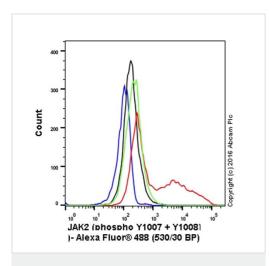
Additional bands at: 60 kDa. We are unsure as to the identity of these extra bands.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

Blocking and diluting buffer: 5% NFDM/TBST

Exposure time:

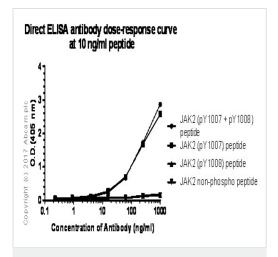
Left image: 1 second Right image: 5 minutes



Flow Cytometry (Intracellular) - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells starved of serum for 16 hours then treated with 1 mM Pervanadate for 30 minutes labeling JAK2 (phospho Y1007 + Y1008) with ab32101 at 1/20 dilution (10 ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor®488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control. Unstimulated Jurkat cells were used as a negative control (Green).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

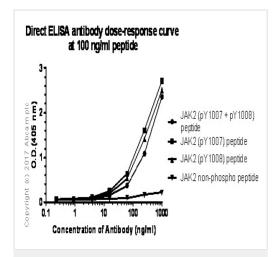


ELISA - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

Direct ELISA antigen dose-response curve using <u>ab32101</u> at 0~1000 ng/mL. Antigen concentration of 10 ng/mL. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit lgG(H+L) (1/2500) was used as the secondary antibody.

This antibody preferentially recognizes phospho Y1007. When the concentration of peptides is lower than 10 ng/mL, it cannot recognize phospho Y1008.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).

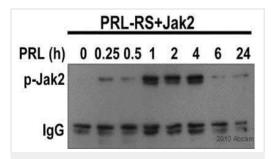


ELISA - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

Direct ELISA antigen dose-response curve using <u>ab32101</u> at 0~1000 ng/mL. Antigen concentration of 100 ng/mL. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit lgG(H+L) (1/2500) was used as the secondary antibody.

This antibody preferentially recognizes phospho Y1007. When the concentration of peptides is higher than 100 ng/mL, it also recognizes phospho Y1008.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] - BSA and Azide free (ab219728)

All lanes : Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) at 1/2000 dilution

Lane 1: Rat uterine cell line - whole cell lysate. Treated with 1µg/mL Prolactin for 0 hours

Lane 2 : Rat uterine cell line - whole cell lysate. Treated with $1\mu g/mL$ Prolactin for 15 minutes

Lane 3: Rat uterine cell line - whole cell lysate. Treated with 1µg/mL Prolactin for 30 minutes

Lane 4: Rat uterine cell line - whole cell lysate. Treated with 1µg/mL Prolactin for 1 hour.

Lane 5 : Rat uterine cell line - whole cell lysate. Treated with $1\mu g/mL$ Prolactin for 2 hours

Lane 6: Rat uterine cell line - whole cell lysate. Treated with 1µg/mL Prolactin for 4 hours

Lane 7: Rat uterine cell line - whole cell lysate. Treated with

1µg/mL Prolactin for 6 hours

Lane 8: Rat uterine cell line - whole cell lysate. Treated with

1µg/mL Prolactin for 24 hours

Lysates/proteins at 30 µg per lane.

Secondary

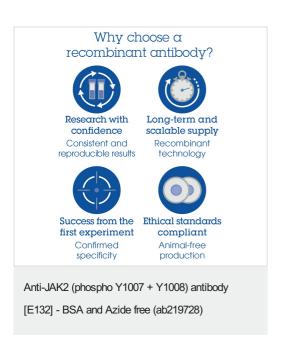
All lanes: HRP-conjugated donkey anti-rabbit polyclonal at

1/10000 dilution

Predicted band size: 130 kDa **Observed band size:** 110 kDa

Additional bands at: 55 kDa (possible non-specific binding)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32101).



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