# abcam

## Product datasheet

## Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] ab32101

Recombinant RabMAb

★★★★★ 4 Abreviews 171 References 12 Images

#### Overview

**Product name** Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132]

**Description** Rabbit monoclonal [E132] to JAK2 (phospho Y1007 + Y1008)

**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: ELISA, Flow Cyt (Intra), WB, ICC/IF, IHC-P, Dot blot

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 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

**Clonality** Monoclonal

Clone number E132
Isotype IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab32101 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	
ELISA		Use at an assay dependent concentration.	
Flow Cyt (Intra)		1/20.	
WB	★★☆☆☆ ( <u>3</u> )	1/1000 - 1/10000. Detects a band of approximately 120 kDa (predicted molecular weight: 130 kDa).  The samples may require stimulation (E.g., Jurkat cells treated with pervanadate for 5 min)	
ICC/IF	<b>★★★★☆ (1)</b>	1/1000.	
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.  The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.	
Dot blot		1/1000.	

#### **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.

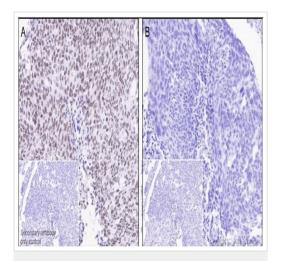
# Post-translational modifications

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.

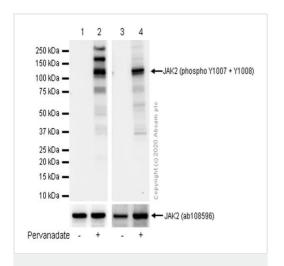
#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101)

ab32101 showing positive staining in Human differentiated squamous cell carcinoma of the cervix tissue at 1/10000 dilution. Goat Anti-Rabbit IgG H&L (HRP) was used as secondary antibody. Antigen retreival was carried out by Heat mediated antigen retrieval using <a href="mailto:ab93684">ab93684</a> (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] (ab32101)

**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  $100~\mu M$  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  $\mu$ M pervanadate for 30 minutes whole cell lysate

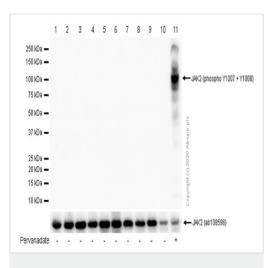
Lysates/proteins at 15 µg per lane.

#### Secondary

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

Predicted band size: 130 kDa

The extra bands are undefined.



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101)

**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

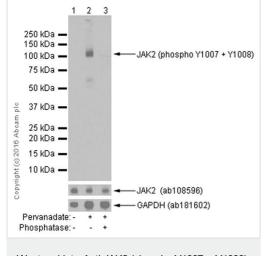
pervanadate for 5 minutes whole cell lysate

Lysates/proteins at 15 µg per lane.

## Secondary

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

Predicted band size: 130 kDa



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (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 lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

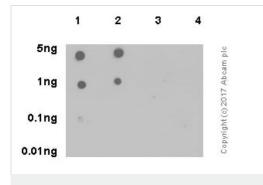
**Predicted band size:** 130 kDa **Observed band size:** 120 kDa

Exposure time: 5 seconds

Blocking and diluting buffer 5% NFDM/TBST

Untreated Jurkat	Ab32101 Anti-JAK2 (phospho Y1007 + Y1008)		Ab108596 Anti-JAK2	
	Ab32101	Ab32101+DAPI+tubulin	Ab108596	Ab108596+DAPI+tubul
Jurkat+Per	0000			
Jurkat+Per +LP				Copyright (c) 2017 Absam p

Immunocytochemistry/ Immunofluorescence - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) 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).

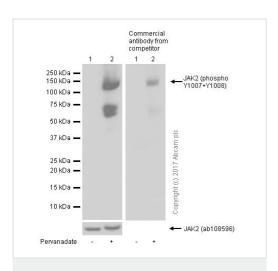


Dot Blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101)

Dot blot analysis of human 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. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051)

was used as the secondary antibody at a dilution of 1/20,000.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (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 lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

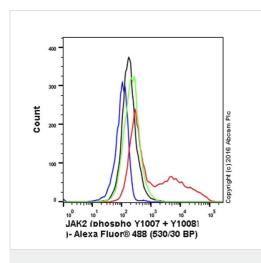
Additional bands at: 60 kDa. We are unsure as to the identity of

these extra bands.

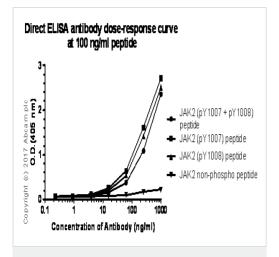
Blocking and diluting buffer: 5% NFDM/TBST

**Exposure time:** 

Left image: 1 second Right image: 5 minutes

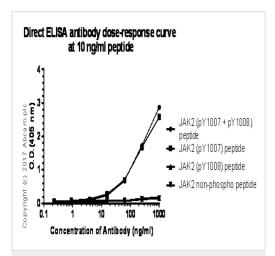


Flow Cytometry (Intracellular) - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells starved of serum for 16 hours then treated with 1 mM Pervanadate for 30 minutes labeling JAK2 (phospho Y1007 + Y1008) with ab32101 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 Fluorr®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).



ELISA - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101) Direct ELISA antigen dose-response curve using ab32101 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.

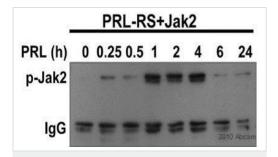
This antibody preferentially recognizes phospho Y1007. When the concentration of peptides is higher than 100 ng/mL, it also recognizes phospho Y1008.



ELISA - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101)

Direct ELISA antigen dose-response curve using ab32101 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.



Western blot - Anti-JAK2 (phospho Y1007 + Y1008) antibody [E132] (ab32101)

This image is courtesy of an anonymous Abreview

**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 1µg/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 1µg/mL Prolactin for 2 hours.

**Lane 6 :** Rat uterine cell line - whole cell lysate. Treated with  $1\mu g/mL$  Prolactin for 4 hours.

**Lane 7 :** Rat uterine cell line - whole cell lysate. Treated with  $1\mu g/mL$  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

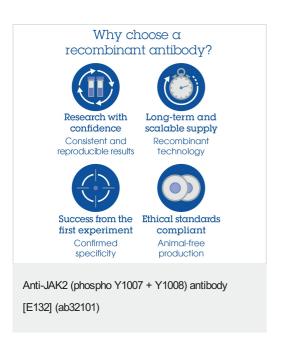
**All lanes :** An HRP-conjugated donkey anti-rabbit polyclonal. at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

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

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



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