abcam

Product datasheet

Anti-c-Kit (phospho Y823) antibody ab5634

1 References 2 Images

Overview

Product name	Anti-c-Kit (phospho Y823) antibody
Description	Rabbit polyclonal to c-Kit (phospho Y823)
Host species	Rabbit
Tested applications	**Suitable for:** ICC, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Chicken, Cow, Dog
Immunogen	Synthetic peptide corresponding to c-Kit (phospho Y823).
Positive control	WB: M07e cells +/- SCF. ICC: Jurkat cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.3 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA
Purity	Immunogen affinity purified
Purification notes	The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated c Kit protein. The final product is generated by affinity chromatography using a c Kit derived peptide that is phosphorylated at tyrosine 823.
Clonality	Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5634 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		1/250.
WB		1/1000. Detects a band of approximately 145 kDa.

Target

Function Tyrosine-protein kinase that acts as cell-surface receptor for the cytokine KITLG/SCF and plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration and function, and in melanogenesis. In response to KITLG/SCF binding, KIT can activate several signaling pathways. Phosphorylates PIK3R1, PLCG1, SH2B2/APS and CBL. Activates the AKT1 signaling pathway by phosphorylation of PIK3R1, the regulatory subunit of phosphatidylinositol 3-kinase. Activated KIT also transmits signals via GRB2 and activation of RAS, RAF1 and the MAP kinases MAPK1/ERK2 and/or MAPK3/ERK1. Promotes activation of STAT family members STAT1, STAT3, STAT5A and STAT5B. Activation of PLCG1 leads to the production of the cellular signaling molecules diacylglycerol and inositol 1,4,5-trisphosphate. KIT signaling is modulated by protein phosphatases, and by rapid internalization and degradation of the receptor. Activated KIT promotes phosphorylation of the protein phosphatases PTPN6/SHP-1 and PTPRU, and of the transcription factors STAT1, STAT3, STAT5A and STAT5B. Promotes phosphorylation of PIK3R1, CBL, CRK (isoform Crk-II), LYN, MAPK1/ERK2 and/or MAPK3/ERK1, PLCG1, SRC and SHC1.

Tissue specificity Isoform 1 and isoform 2 are detected in spermatogonia and Leydig cells. Isoform 3 is detected in round spermatids, elongating spermatids and spermatozoa (at protein level). Widely expressed. Detected in the hematopoietic system, the gastrointestinal system, in melanocytes and in germ cells.

Involvement in disease Piebald trait
Gastrointestinal stromal tumor
Testicular germ cell tumor
Leukemia, acute myelogenous

Sequence similarities Belongs to the protein kinase superfamily. Tyr protein kinase family. CSF-1/PDGF receptor subfamily.
Contains 5 Ig-like C2-type (immunoglobulin-like) domains.
Contains 1 protein kinase domain.

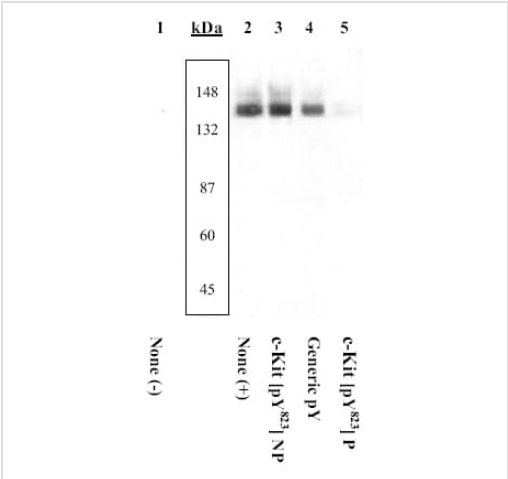
Post-translational modifications Ubiquitinated by SOCS6. KIT is rapidly ubiquitinated after autophosphorylation induced by KITLG/SCF binding, leading to internalization and degradation.
Autophosphorylated on tyrosine residues. KITLG/SCF binding enhances autophosphorylation. Isoform 1 shows low levels of tyrosine phosphorylation in the absence of added KITLG/SCF (in vitro). Kinase activity is down-regulated by phosphorylation on serine residues by protein kinase C family members. Phosphorylation at Tyr-568 is required for interaction with PTPN11/SHP-2,

CRK (isoform Crk-II) and members of the SRC tyrosine-protein kinase family. Phosphorylation at Tyr-570 is required for interaction with PTPN6/SHP-1. Phosphorylation at Tyr-703, Tyr-823 and Tyr-936 is important for interaction with GRB2. Phosphorylation at Tyr-721 is important for interaction with PIK3R1. Phosphorylation at Tyr-823 and Tyr-936 is important for interaction with GRB7.

Cellular localization

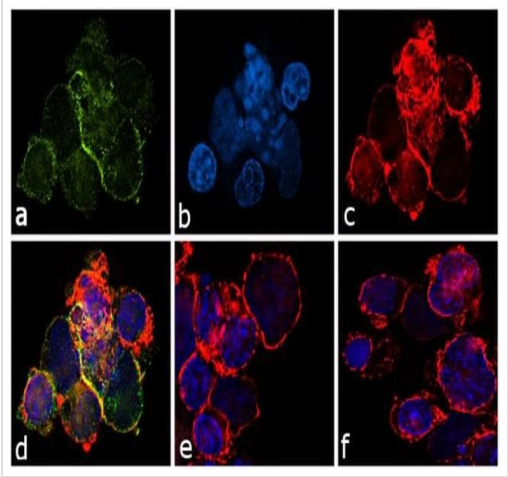
Cell membrane and Cytoplasm. Detected in the cytoplasm of spermatozoa, especially in the equatorial and subacrosomal region of the sperm head.

Images



Western blot - Anti-c-Kit (phospho Y823) antibody (ab5634)

Peptide Competition: Extracts prepared from M07e cells left untreated (1) or treated (2-5) with SCF were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4°C, then were incubated with 0.50 µg/mL ab5634 antibody for two hours at room temperature in a 1% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphotyrosine containing peptide (4), or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and signals were detected using the Tropix WesternStar method. The data show that only the peptide corresponding to ab5634 blocks the antibody signal, thereby demonstrating the specificity of the antibody. Peptide Competition: Extracts prepared from M07e cells left untreated (1) or treated (2-5) with SCF were resolved



Immunocytochemistry - Anti-c-Kit (phospho Y823) antibody (ab5634)

Jurkat cells stained for c-Kit cleave site (green) using ab5634 at 1/250 dilution in ICC/IF. It was followed by Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at 1/2000 dilution for 45 minutes at room temperature (Panel a). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c: red) was stained with Rhodamine Phalloidin at 1/300 dilution. Panel d represents the merged image showing membranous localization. Panel e shows the untreated control cells with no signal. Panel f shows the no primary antibody control.

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