# abcam

# Product datasheet

# Anti-c-Kit antibody [YR145] ab32363





★★★★★ 10 Abreviews 50 References

11 Images

#### Overview

**Product name** Anti-c-Kit antibody [YR145]

**Description** Rabbit monoclonal [YR145] to c-Kit

**Host species** Rabbit

Specificity This antibody is specific to c-Kit. It does not crossreact with other CSF1/PDGF receptor family

Suitable for: IHC-P, WB **Tested applications** 

Unsuitable for: Flow Cyt or ICC/IF

Reacts with: Human, Common marmoset Species reactivity

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

Positive control WB: Human fetal kidney, fetal lung and seminoma tissue lysate. IHC-P: Human lung

adenocarcinoma and seminoma tissues.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

**Properties** 

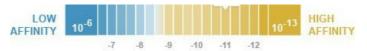
**Form** Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Storage instructions

Avoid freeze / thaw cycle.

 $K_D = 5.90 \times 10^{-11} M$ Dissociation constant (K<sub>D</sub>)

10<sup>-11</sup>



# Learn more about K<sub>D</sub>

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

**Clonality** Monoclonal

Clone number YR145

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab32363 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
IHC-P	<b>★★★★★</b> (4)	1/400. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.
WB	<b>★★★★★ (1)</b>	1/1000. Predicted molecular weight: 110 kDa.

**Application notes** Is unsuitable for Flow Cyt or ICC/IF.

#### **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 lg-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-936 is important for interaction with

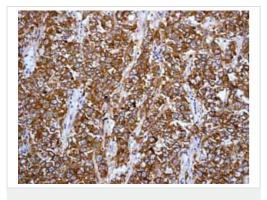
GRB7.

#### **Cellular localization**

 $\label{lem:continuous} \textbf{Cell membrane and Cytoplasm}. \textbf{ Detected in the cytoplasm of spermatozoa, especially in the }$ 

equatorial and subacrosomal region of the sperm head.

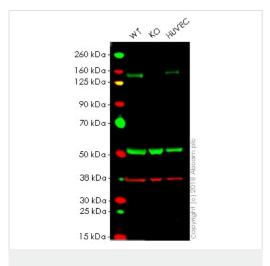
#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Kit antibody [YR145] (ab32363)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human seminoma tissue labelling c-Kit with unpurified ab32363.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-c-Kit antibody [YR145] (ab32363)

All lanes: Anti-c-Kit antibody [YR145] (ab32363) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: KIT knockout HAP1 whole cell lysate

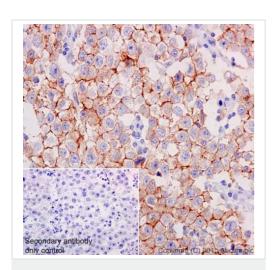
Lane 3: HUVEC whole cell lysate

Lysates/proteins at 40 µg per lane.

Predicted band size: 110 kDa

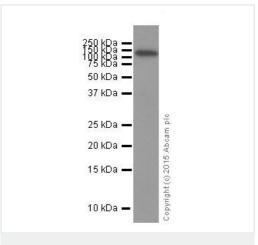
**Lanes 1 - 3:** Merged signal (red and green). Green - ab32363 observed at 109 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab32363 was shown to recognize 0 in wild-type HAP1 cells as signal was lost at the expected MW in KIT knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and KIT knockout samples were subjected to SDS-PAGE. Ab32363 and <a href="mailto:ab9484">ab9484</a> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Kit antibody [YR145] (ab32363)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human seminoma tissue labelling c-Kit with purified ab32363 at 1/400. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-c-Kit antibody [YR145] (ab32363)

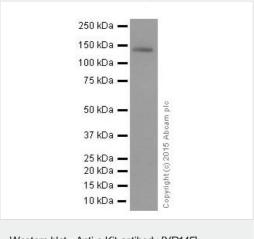
Anti-c-Kit antibody [YR145] (ab32363) at 1/5000 dilution (purified) + Human seminoma tissue lysate at 10  $\mu g$ 

# Secondary

HRP-conjugated anti-rabbit  $\lg G$ , specific to the non-reduced form of  $\lg G$  at 1/50000 dilution

**Predicted band size:** 110 kDa **Observed band size:** 120-145 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Anti-c-Kit antibody [YR145] (ab32363) at 1/1000 dilution (purified) + Human fetal lung tissue lysate at 10 µg

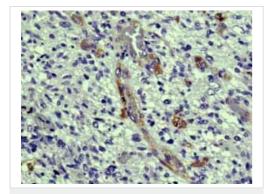
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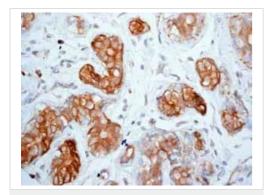
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Kit antibody [YR145] (ab32363)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue labelling c-Kit with unpurified ab32363.

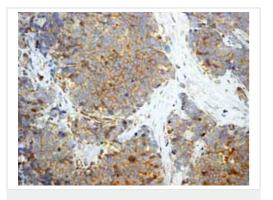
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Kit antibody [YR145] (ab32363)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling c-Kit with unpurified ab32363.

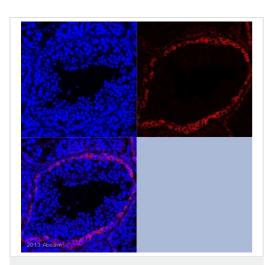
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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Kit antibody [YR145] (ab32363)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian carcinoma tissue labelling c-Kit with unpurified ab32363.

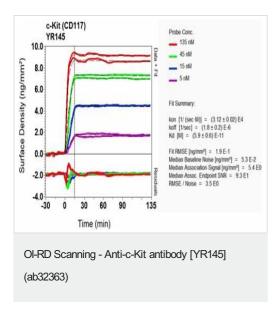
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Kit antibody [YR145] (ab32363)

This image is courtesy of an abreview from Zachary Yu-Ching Lin.

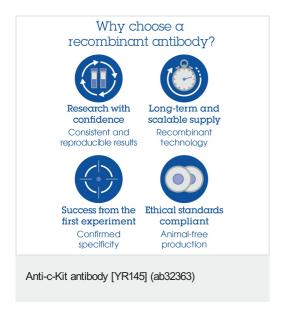
IHC-P image of c-Kit (unpurified ab32363) staining on adult marmoset testis. The sections were fixed in formaldehyde and underwent heat mediated antigen retrieval, using Dako antigen retrieval solution. The sections were then blocked in 5% milk for 30 mins at 25°C. The primary antibody was added at a 1/100 dilution, and then incubated for 18 hours at 4°C. Dapi was used to stain the nuclei (blue).



Equilibrium disassociation constant (K<sub>D</sub>)

Learn more about KD

Click here to learn more about K<sub>D</sub>



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