abcam

Anti-c-Kit antibody [EPR25707-134] ab283653

重组 RabMAb®

11 图像

概述

产品名称	Anti-c-Kit抗体[EPR25707-134]
描述	兔单克隆抗体[EPR25707-134] to c-Kit
宿主	Rabbit
经测试应用	**适用于**: WB, IHC-P, Flow Cyt, IP, ICC/IF
种属反应性	**与反应**: Human **不与反应**: Mouse, Rat
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HEL, HL-60, Jurkat and Saos-2 whole cell lysates. IHC-P: Human colon, Human hyperplastic prostate, Human colon cancer and Human breast cancer tissues. ICC/IF: HEL cells. Flow Cyt: HL-60 and HEL cells. IP: Saos-2 whole cell lysate.
常规说明	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR25707-134
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab283653于以下的经测试应用

"**应用说明**"部分 下显示的仅为**推荐的起始稀释度**；实际**最佳的稀释度/浓度应由使用者检定。**

应用	Ab评论	说明
WB		1/1000. Predicted molecular weight: 109 kDa.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		1/500.
IP		1/30.
ICC/IF		1/250.

靶标

功能	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.
组织特异性	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.
疾病相关	Piebald trait Gastrointestinal stromal tumor Testicular germ cell tumor Leukemia, acute myelogenous
序列相似性	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.

翻译后修饰	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.
细胞定位	Cell membrane and Cytoplasm. Detected in the cytoplasm of spermatozoa, especially in the equatorial and subacrosomal region of the sperm head.

图片
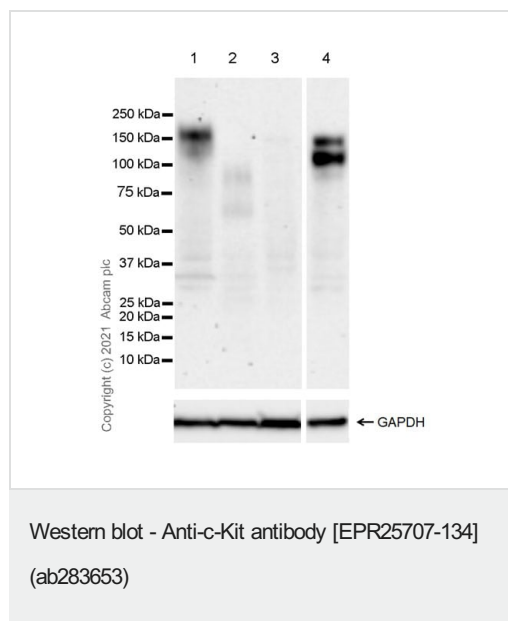
Western blot - Anti-c-Kit antibody [EPR25707-134] (ab283653)

All lanes : Anti-c-Kit antibody [EPR25707-134] (ab283653) at 1/1000 dilution

Lane 1 : HEL (human erythroleukemia erythroblast) whole cell lysate

Lane 2 : HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate

Lane 3 : Jurkat (human T cell leukemia T lymphocyte) whole cell lysate

Lane 4 : Saos-2 (human osteosarcoma epithelial) whole cell lysate

Lysates/proteins at 40 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution
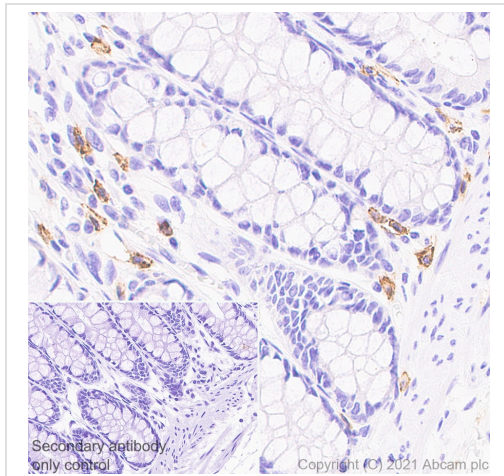
Developed using the ECL technique.

Predicted band size: 109 kDa
Observed band size: 120,140-160 kDa

Exposure time: 125 seconds

Blocking buffer: 5% NFDM/TBST.

Low expression: HL-60 (PMID: 29127384) and Jurkat (PMID: 22140461).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labelling c-Kit with ab283653 at 1/100 dilution followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining in lamina propria cells of human colon (PMID: 23276179). The section was incubated with ab283653 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins
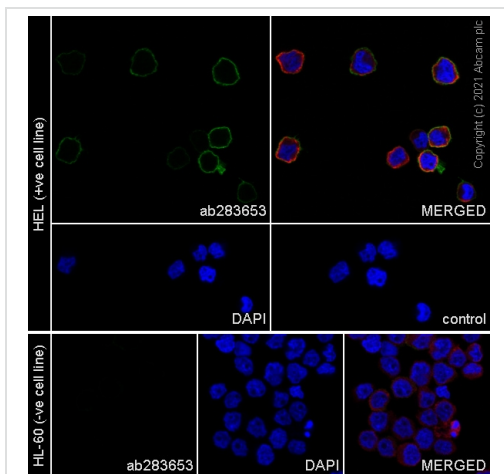
Immunocytochemistry/ Immunofluorescence - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HEL cells labelling c-Kit with ab283653 at 1/250 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Confocal image showing membranous staining in HEL cells. Negative control: HL-60 (PMID: 29127384) is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.
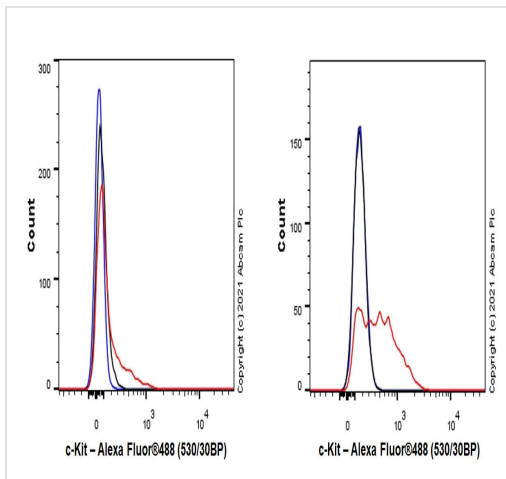
Flow Cytometry - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Flow cytometric analysis of HL-60 (Human Acute Promyelocytic Leukemia promyeloblast, Left)/ Saos-2 (human osteosarcoma epithelial, Right) cells labelling c-Kit with ab283653 at 1/500 (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat F(ab')2 Anti-Rabbit IgG(DyLight$^{®}$ 488, **ab98507**) at 1/500 was used as the secondary antibody. Negative control: HL-60 (PMID: 29127384). Gated on viable cells.
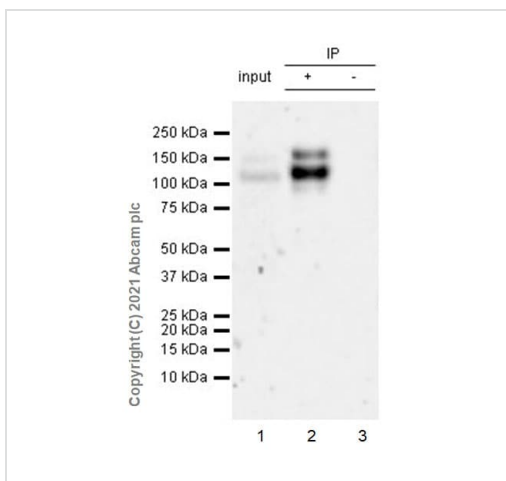
Immunoprecipitation - Anti-c-Kit antibody [EPR25707-134] (ab283653)

c-Kit was immunoprecipitated from 0.35 mg Saos-2 (human osteosarcoma epithelial) whole cell lysate 10 μg with ab283653 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab283653 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.
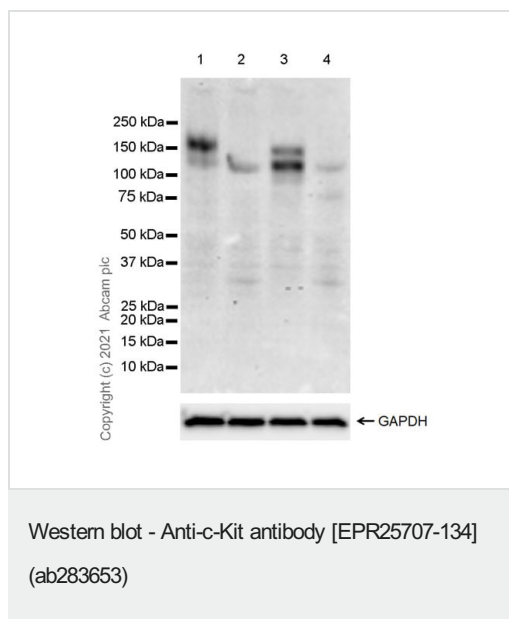
Lane 1: Saos-2 (human osteosarcoma epithelial) whole cell lysate 10 μg

Lane 2: Saos-2 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab283653 in Saos-2 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes

Western blot - Anti-c-Kit antibody [EPR25707-134] (ab283653)

All lanes : Anti-c-Kit antibody [EPR25707-134] (ab283653) at 1/1000 dilution

Lane 1 : HEL (human erythroleukemia erythroblast) whole cell lysate

Lane 2 : HEL whole cell lysate treated with PNGase F

Lane 3 : Saos-2 (human osteosarcoma epithelial) whole cell lysate

Lane 4 : Saos-2 whole cell lysate treated with PNGase F

Lysates/proteins at 20 μg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Developed using the ECL technique.
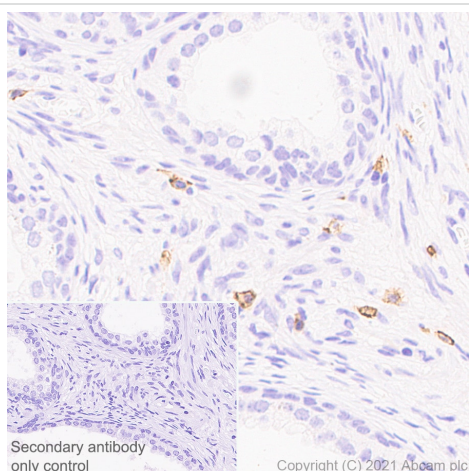
Predicted band size: 109 kDa

Observed band size: 120,140-160 kDa

Exposure time: 3 minutes

Blocking buffer: 5% NFDM/TBST.

The difference in target band size observed in HEL and Saos-2 cell lysates is caused by glycosylation.

This blot was developed using a higher sensitivity ECL substrate.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Immunohistochemical analysis of paraffin-embedded Human hyperplastic prostate tissue labelling c-Kit with ab283653 at 1/100 dilution followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on stromal cells of human hyperplastic prostate (PMID: 23276179). The section was incubated with ab283653 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins
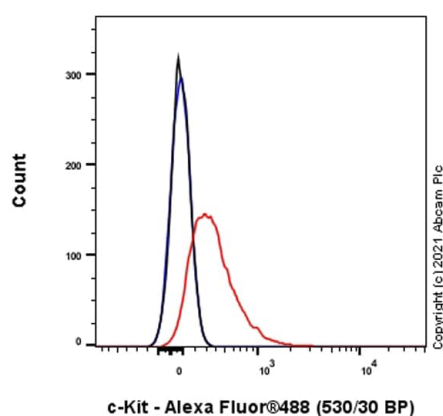
Flow Cytometry - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Flow cytometric analysis of HEL (human erythroleukemia erythroblast) cells labelling c-Kit with ab283653 (Red) at 1/500 dilution compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat F(ab')2 Anti-Rabbit IgG(DyLight® 488, **ab98507**) at 1/500 dilution was used as the secondary antibody. Gated on viable cells.
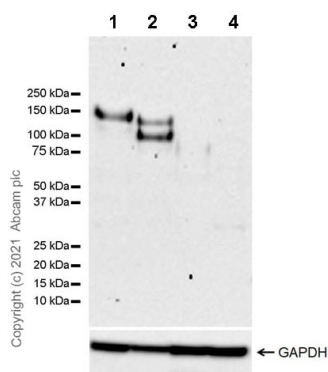
Western blot - Anti-c-Kit antibody [EPR25707-134] (ab283653)

All lanes : Anti-c-Kit antibody [EPR25707-134] (ab283653) at 1/1000 dilution

Lane 1 : HEL (human erythroleukemia erythroblast) whole cell lysate

Lane 2 : Saos-2 (human osteosarcoma epithelial) whole cell lysate

Lane 3 : HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate

Lane 4 : Jurkat (human T cell leukemia T lymphocyte) whole cell lysate

Lysates/proteins at 40 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Developed using the ECL technique.
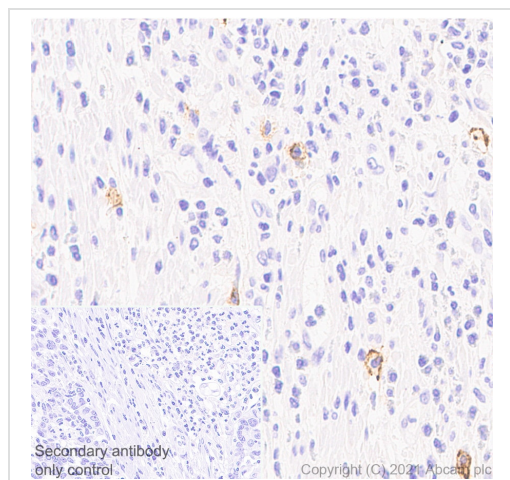
Predicted band size: 109 kDa

Observed band size: 120,140-160 kDa

Exposure time: 3 minutes

Blocking buffer: 5% NFDM/TBST.

Low expression: HL-60 (PMID: 29127384) and Jurkat (PMID: 22140461).
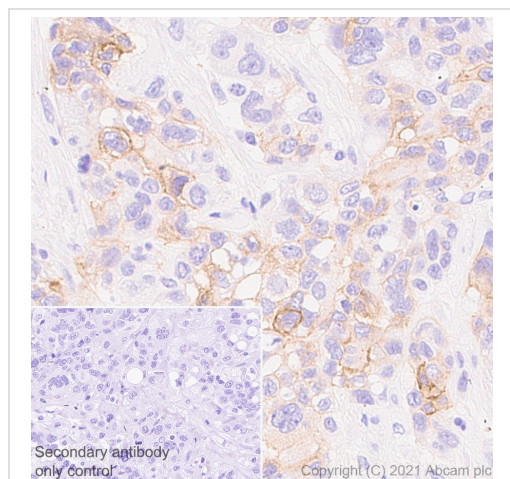
Bis-tris gel was used in the blot.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labelling c-Kit with ab283653 at 1/100 dilution followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on stromal cells of human colon cancer (PMID: 23276179). The section was incubated with ab283653 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Kit antibody [EPR25707-134] (ab283653)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labelling c-Kit with ab283653 at 1/100 dilution followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human breast cancer. The section was incubated with ab283653 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

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