abcam

Product datasheet

Anti-SHP2 antibody [Y478] ab32083

KO 評価済 リコンビナント RabMAb

12 References 画像数 13

製品の概要

製品名	Anti-SHP2 antibody [Y478]
製品の詳細	Rabbit monoclonal [Y478] to SHP2
由来種	Rabbit
特異性	This antibody recognises SHP2. This antibody is predicted to detect splice isoform 2 based on sequence analysis.
アプリケーション	**適用あり:** WB, IHC-P, IP, Flow Cyt (Intra), ICC/IF
種交差性	**交差種:** Human
免疫原	Synthetic peptide within Human SHP2 aa 500-600 (C terminal). The exact sequence is proprietary.
ポジティブ・コントロール	IHC-P: Human breast carcinoma and endometrium tissue. WB: HEK-293T, Jurkat, and THP-1 cell lysate. ICC/IF: Hek293 and A431 cells. Flow Cyt (intra): HAP1-WT and Jurkat cells IP: THP-1 whole cell lysate
特記事項	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.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y478
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab32083の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000 - 1/10000. Predicted molecular weight: 68 kDa.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/40. **For unpurified use at 1/50**
Flow Cyt (Intra)		1/50. For unpurified use at 0.1 µg/ml
ICC/IF		1/50. **For unpurified use at 1/100.**

ターゲット情報

機能	Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus.
組織特異性	Widely expressed, with highest levels in heart, brain, and skeletal muscle.
関連疾患	Defects in PTPN11 are the cause of LEOPARD syndrome type 1 (LEOPARD1) [MIM:151100]. It is an autosomal dominant disorder allelic with Noonan syndrome. The acronym LEOPARD stands for lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormalities of genitalia, retardation of growth, and deafness. Defects in PTPN11 are the cause of Noonan syndrome type 1 (NS1) [MIM:163950]. Noonan syndrome (NS) is a disorder characterized by dysmorphic facial features, short stature, hypertelorism, cardiac anomalies, deafness, motor delay, and a bleeding diathesis. Some patients with Noonan syndrome type 1 develop multiple giant cell lesions of the jaw or other bony or soft tissues, which are classified as pigmented villomoduolar synovitis (PVNS) when occurring in the jaw or joints. Note=Mutations in PTPN11 account for more than 50% of the cases. Rarely, NS is associated with juvenile myelomonocytic leukemia (JMML). NS1 inheritance is autosomal dominant. Defects in PTPN11 are a cause of juvenile myelomonocytic leukemia (JMML) [MIM:607785]. JMML is a pediatric myelodysplastic syndrome that constitutes approximately 30% of childhood cases of myelodysplastic syndrome (MDS) and 2% of leukemia. It is characterized by leukocytosis with tissue infiltration and in vitro hypersensitivity of myeloid progenitors to granulocyte-macrophage colony stimulating factor.

Defects in PTPN11 are a cause of metachondromatosis (MC) [MIM:156250]. It is a skeletal disorder with radiologic fetarures of both multiple exostoses and Ollier disease, characterized by the presence of multiple enchondromas and osteochondroma-like lesions.

配列類似性

Belongs to the protein-tyrosine phosphatase family. Non-receptor class 2 subfamily.
Contains 2 SH2 domains.
Contains 1 tyrosine-protein phosphatase domain.

ドメイン

The SH2 domains repress phosphatase activity. Binding of these domains to phosphotyrosine-containing proteins relieves this auto-inhibition, possibly by inducing a conformational change in the enzyme.

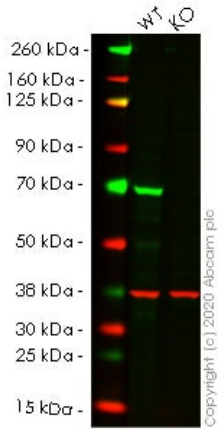
翻訳後修飾

Phosphorylated on Tyr-546 and Tyr-584 upon receptor protein tyrosine kinase activation; which creates a binding site for GRB2 and other SH2-containing proteins.

細胞内局在

Cytoplasm.

画像



Western blot - Anti-SHP2 antibody [Y478] (ab32083)

All lanes : Anti-SHP2 antibody [Y478] (ab32083) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PTPN11 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

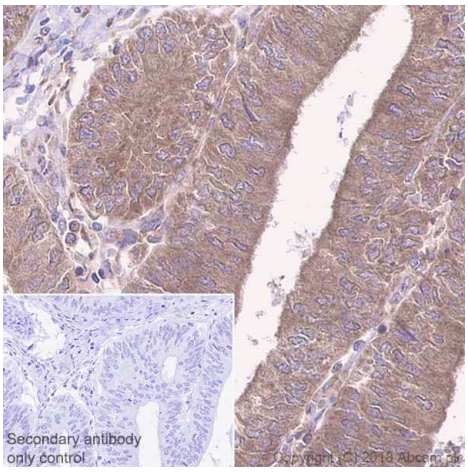
Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 68 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab32083 observed at 68 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

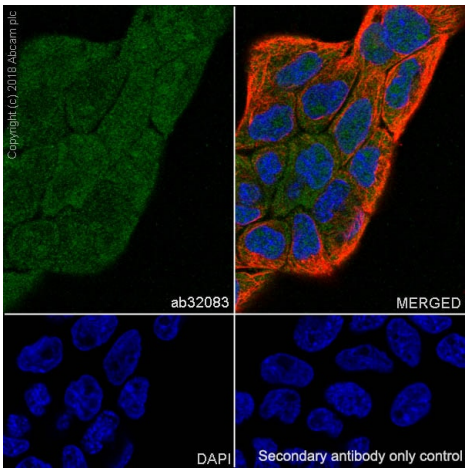
ab32083 was shown to react with SHP2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266450** (knockout cell lysate **ab257618**) was used. Wild-type HEK-293T and PTPN11 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32083 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room

temperature before imaging.



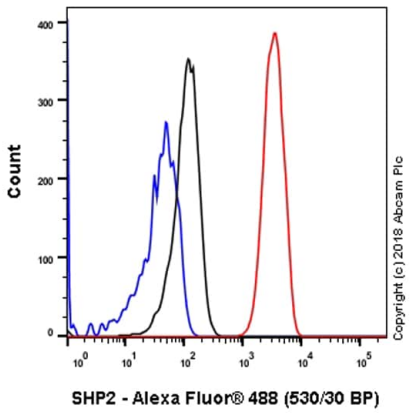
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SHP2 antibody [Y478] (ab32083)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human endometrium cancer tissue sections labeling SHP2 with Purified ab32083 at 1:100 dilution (5.51 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)ImmunoHistoProbe one step HRP Polymer (ready to use)was used as the secondary antibody.Negative control:PBS instead of the primary antibody.Hematoxylinwas used as a counterstain



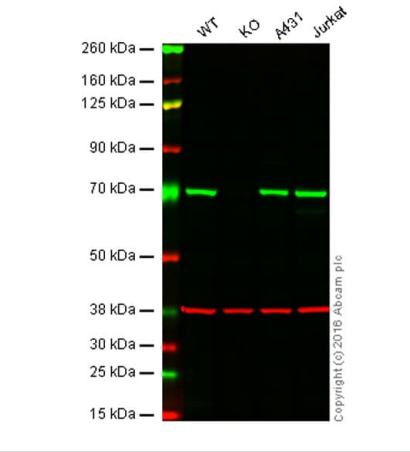
Immunocytochemistry/ Immunofluorescence - Anti-SHP2 antibody [Y478] (ab32083)

Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling SHP2 with Purified ab32083 at 1:50 dilution (11 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor®594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor®488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



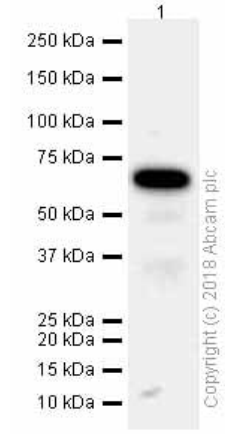
Flow Cytometry (Intracellular) - Anti-SHP2 antibody [Y478] (ab32083)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling SHP2 with purified ab32083 at 1/50 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-SHP2 antibody [Y478] (ab32083)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SHP2 knockout HAP1 cell lysate (20 µg)
Lane 3: A431 cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)
Lanes 1 to 4: Merged signal (red and green). Green - ab32083 observed at 68 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
unpurified ab32083 was shown to specifically react with SHP2 when SHP2 knockout samples were used. Wild-type and SHP2 knockout samples were subjected to SDS-PAGE. ab32083 and **ab8245** (loading control to GAPDH) were both diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-SHP2 antibody [Y478] (ab32083)

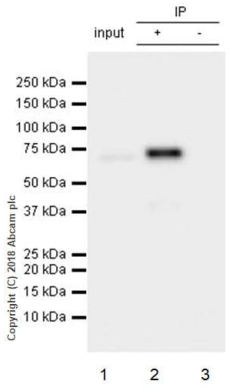
Anti-SHP2 antibody [Y478] (ab32083) at 0.3 µg/ml (purified) + THP-1 (Human monocytic leukemia monocyte) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

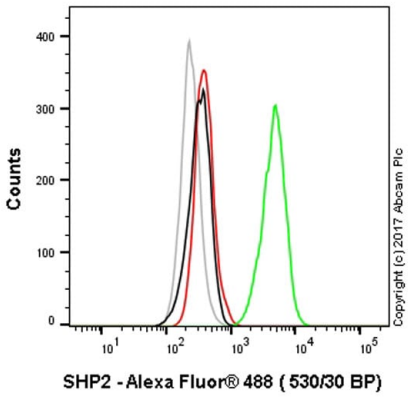
Predicted band size: 68 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-SHP2 antibody [Y478] (ab32083)

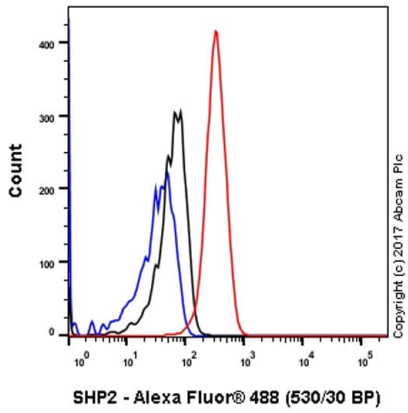
ab32083 (purified) at 1:40 dilution (2µg) immunoprecipitating SHP2 in THP-1 whole cell lysate.
Lane 1 (input): THP-1 (Human monocytic leukemia monocyte) whole cell lysate 10µg
Lane 2 (+): ab32083 & THP-1 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32083 in THP-1 whole cell lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-SHP2 antibody [Y478] (ab32083)

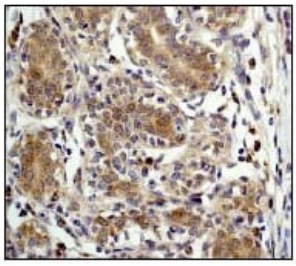
Overlay histogram showing HAP1 wildtype (green line) and HAP1-PTPN11 knockout cells (red line) stained with ab32083. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (unpurified ab32083, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-PTPN11 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 80%

methanol (5 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



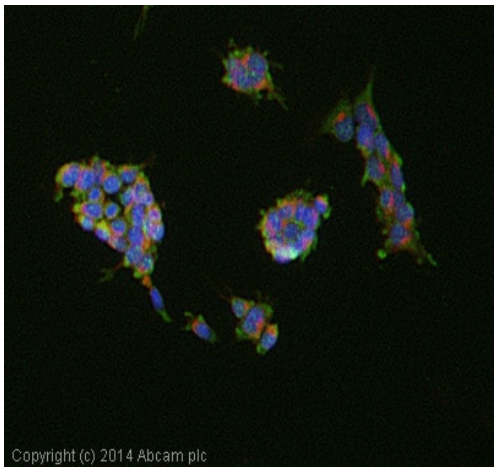
Flow Cytometry (Intracellular) - Anti-SHP2 antibody [Y478] (ab32083)

Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling SHP2 with unpurified ab32083 at 1/500 dilution (1ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) (**ab172730**) was used as the isotype control, Cell without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



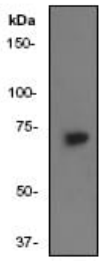
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SHP2 antibody [Y478] (ab32083)

Immunohistochemical analysis of SHP2 expression in paraffin embedded human breast carcinoma, using 1/50 unpurified ab32083.



Immunocytochemistry/ Immunofluorescence - Anti-SHP2 antibody [Y478] (ab32083)

unpurified ab32083 stained Hek293 cells. The cells were 100% methanol fixed for 5 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32083 at 1/100 dilution) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-SHP2 antibody [Y478] (ab32083)

Anti-SHP2 antibody [Y478] (ab32083) at 1/5000 dilution (unpurified) + Jurkat cell lysate

Predicted band size: 68 kDa
Observed band size: 70 kDa

Anti-SHP2 antibody [Y478] (ab32083)

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