abcam

Product datasheet

Anti-SHP2 antibody [EPR26539-45] ab300579



ייבעדין RabMAb

画像数 13

製品の概要

製品名 Anti-SHP2 antibody [EPR26539-45]

製品の詳細 Rabbit monoclonal [EPR26539-45] to SHP2

由来種 Rabbit

アプリケーション 適用あり: WB, ICC/IF, IHC-P, IP

適用なし: Flow Cyt (Intra)

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Wild-type HEK293T, K562 whole cell lysates; human heart and cerebellum tissue lysates;

> mouse brain and heart tissue lysates; rat brain and heart tissue lysates; NIH/3T3 whole cell lysate. ICC/IF: Wildtype HEK293T cell line; NIH/3T3 cell line. IP: NIH/3T3 and HeLa whole cell lysate. IHC-

P: Human tonsil, mouse testis, rat testis FFPE tissue sections; Wild-type HEK293T cells.

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**.

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 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

EPR26539-45

アイソタイプ

lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab300579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. Detects a band of approximately 72 kDa.
ICC/IF		1/50.
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

追加情報

Is unsuitable for Flow Cyt (Intra).

ターゲット情報

機能

組織特異性

関連疾患

Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus.

Widely expressed, with highest levels in heart, brain, and skeletal muscle.

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

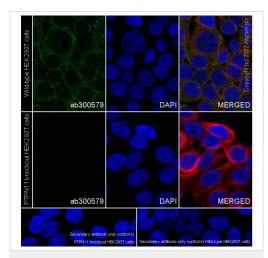
the enzyme.

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



Immunocytochemistry/ Immunofluorescence - Anti-SHP2 antibody [EPR26539-45] (ab300579)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized PTPN11 KO HEK293T (PTPN11 KO knock out human embryonic kidney epithelial cell) (ab266450) cells labeling SHP2 with ab300579 at 1/50 (9.76 µg/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 µg/ml) dilution (Green). Confocal image showing cytoplasmic and weak nuclear staining in wildtype HEK293T cell line, while showing no staining in PTPN11 knockout HEK293T cell line is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 (2.5 µg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at

1/1000 (2 µg/ml) dilution.

250 kDa-150 kDa= 100 kDa= 75 kDa 50 kDa 37 kDa= 2022 15 kDa= 10 kDa=

Western blot - Anti-SHP2 antibody [EPR26539-45] (AB300579)

All lanes: Anti-SHP2 antibody [EPR26539-45] (ab300579) at 1/1000 dilution

Lane 1: Wild-type HEK293T (human embryonic kidney epithelial cell), whole cell lysate

Lane 2: PTPN11 (SHP2) knockout HEK293T whole cell lysate

Lane 3: K562 (human chronic myelogenouseukemia lymphoblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 72 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST Lysates at 20 µg per lane.

In Western blot, ab300579 was shown to bind specifically to PTPN11 (SHP2). A band was observed at 72 kDa in wild-type HEK293T cell lysates with no signal observed at this size in PTPN11 (SHP2) knockout cell line <u>ab266450</u> (knockout cell lysate <u>ab257618</u>).

Exposure time: 180 seconds

Anti-SHP2 antibody [EPR26539-45] (ab300579) at 1/1000 dilution + NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 72 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

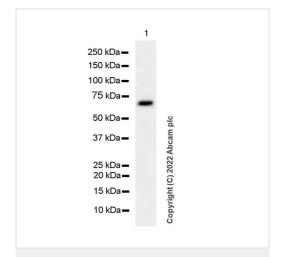
Lysate was freshly made and used for Western blotting immediately to minimize protein degradation.

Exposure time: 48 seconds

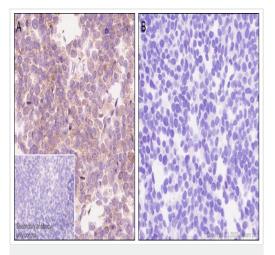
Immunohistochemical analysis of paraffin-embedded (A) Wild-type HEK293 tissue labeling SHP2 with ab300579 at 1/200 (1.22 μ g/mL) followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP polymer). Positive staining in wild-type HEK293T cells and no staining in PTPN11 (SHP2) knockout HEK293T cells. The section was incubated with ab300579 at 4°C overnight. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP polymer).

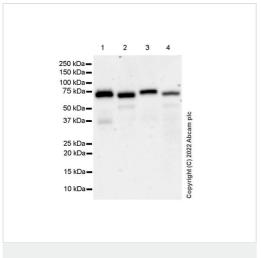
Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0)



Western blot - Anti-SHP2 antibody [EPR26539-45] (AB300579)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHP2 antibody
[EPR26539-45] (AB300579)



Western blot - Anti-SHP2 antibody [EPR26539-45] (AB300579)

All lanes : Anti-SHP2 antibody [EPR26539-45] (ab300579) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate
Lane 2 : Mouse heart tissue lysate
Lane 3 : Rat brain tissue lysate

Lane 4: Rat heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Observed band size: 72 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

This blot was developed using a high sensitivity ECL substrate.

The bands beneath the target band (72 kDa) are likely to be degraded target fragments.

Exposure time: 81 seconds

1 2

250 kDa = 150 kDa = 100 kDa = 75 kDa = 37 kDa = 25 kDa = 20 kDa = 15 kDa = 10 k

Western blot - Anti-SHP2 antibody [EPR26539-45] (AB300579)

All lanes : Anti-SHP2 antibody [EPR26539-45] (ab300579) at 1/1000 dilution

Lane 1: Human cerebellum tissue lysate

Lane 2: Human heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG (Merck DC03L) at 1/2000 dilution

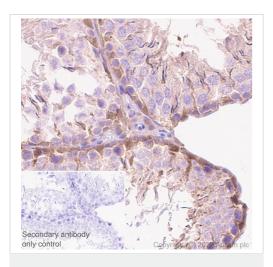
Observed band size: 72 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

This blot was developed using a high sensitivity ECL substrate.

The bands beneath the target band (72 kDa) are likely to be degraded target fragments.

Exposure time: 26 seconds

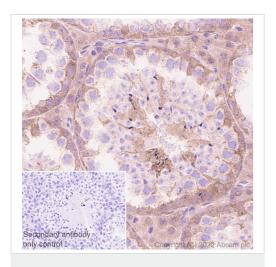


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHP2 antibody
[EPR26539-45] (AB300579)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling SHP2 with ab300579 at 1/50 (9.76 μ g/mL) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer). Positive staining in rat testis (PMID:24123360). The section was incubated with ab300579 at 4°C overnight. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0)

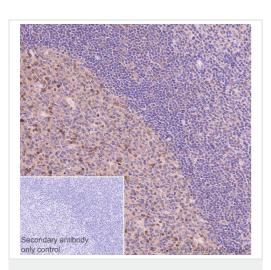


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHP2 antibody
[EPR26539-45] (AB300579)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling SHP2 with ab300579 at 1/50 (9.76 μ g/mL) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer). Positive staining in mouse testis (PMID:24123360). The section was incubated with ab300579 at 4°C overnight. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP polymer).

Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0)

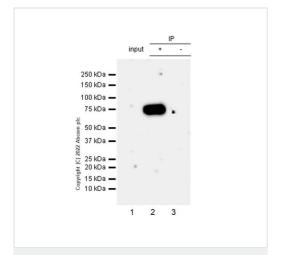


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SHP2 antibody
[EPR26539-45] (AB300579)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling SHP2 with ab300579 at 1/50 (9.76 μ g/mL) followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP polymer). Positive staining in human tonsil (PMID:18728972). The section was incubated with ab300579 at 4°C overnight. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP polymer).

Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0)



Immunoprecipitation - Anti-SHP2 antibody [EPR26539-45] (AB300579)

SHP2 was immunoprecipitated from 0.35 mg HeLa (human cervical adenocarcinoma epithelial cell), whole cell lysate 10 μ g with ab300579 at 1/30 dilution (2 μ g in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300579 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

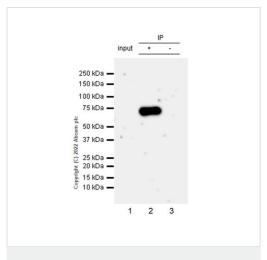
 $\mbox{\bf Lane 1:} \ \mbox{HeLa (human cervical adenocarcinoma epithelial cell),} \label{eq:hela}$ whole cell lysate 10 $\mbox{\sc \mug}$

Lane 2: ab300579 IP in Hela whole cell lysate

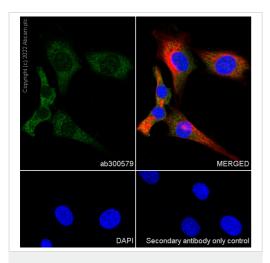
 $\begin{tabular}{ll} \textbf{Lane 3}: Rabbit monoclonal IgG ($\underline{ab172730}$) instead of ab300579 \\ in Hela whole cell lysate \\ \end{tabular}$

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

Exposure time: 180 seconds



Immunoprecipitation - Anti-SHP2 antibody [EPR26539-45] (AB300579)



Immunocytochemistry/ Immunofluorescence - Anti-SHP2 antibody [EPR26539-45] (ab300579)

SHP2 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10µg with ab300579 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300579 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10µq

Lane 2: ab300579 IP in NIH/3T3 whole cell lysate

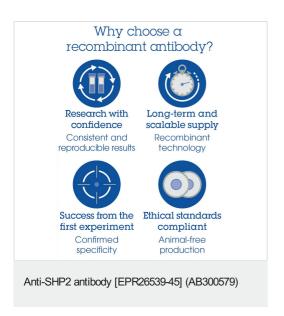
Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab300579 in NIH/3T3 whole cell lysate

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

Exposure time: 180 seconds

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labeling SHP2 with ab300579 at 1/50 (9.76 ug/ml) dilution, followed by <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor 488) preadsorbed antibody at 1/1000 (2 μ g/ml) dilution (Green). Confocal image showing cytoplasmic and weak nuclear staining in NIH/3T3 cell line is observed. <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor 594) was used to counterstain tubulin at 1/200 (2.5 μ g/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 μ g/ml) dilution.



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