abcam

Product datasheet

Anti-Fyn antibody [EPR19636] ab184276





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Overview

Product name Anti-Fyn antibody [EPR19636]

Description Rabbit monoclonal [EPR19636] to Fyn

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, IP, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Human brain lysate; Ramos, HEK-293, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates;

> P0 mouse brain lysate; P0 rat brain lysate; Rat spleen lysate. IHC-P: Human tonsil and cerebral cortex tissues; Mouse spleen tissue; Rat testis tissue. Flow Cyt (intra): RAW 264.7 cells. IP: P0

mouse brain lysate.

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**.

Properties

Form

Storage instructions 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.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number **EPR19636**

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab184276 in the following tested applications.

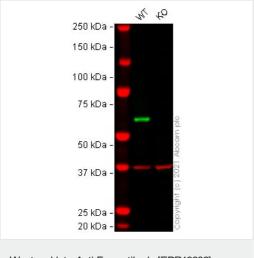
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 61 kDa (predicted molecular weight: 61 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/40.
Flow Cyt (Intra)		1/50.

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Function	Tyrosine-protein kinase implicated in the control of cell growth. Plays a role in the regulation of intracellular calcium levels, with isoform 2 showing the greater ability to mobilize cytoplasmic calcium in comparison to isoform 1. Required in brain development and mature brain function with important roles in the regulation of axon growth, axon guidance, and neurite extension. Blocks axon outgrowth and attraction induced by NTN1 by phosphorylating its receptor DDC. Phosphorylates RUNX3.
Tissue specificity	Isoform 1 is highly expressed in the brain. Isoform 2 is expressed in cells of hemopoietic lineages, especially T lymphocytes.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. SRC subfamily. Contains 1 protein kinase domain. Contains 1 SH2 domain. Contains 1 SH3 domain.
Cellular localization	Cell membrane. Present and active in lipid rafts. Present in cell body and along the process of mature and developing oligodendroyctes.
Form	This protein is known to be similar in amino acid sequence to HCK (P08631), LCK (P06239), YES1 (P07947), SRC (P12931), and LYN (P07948). Therefore, cross-reactivity with these homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request.

Images



Western blot - Anti-Fyn antibody [EPR19636] (ab184276)

Lane 1: Wild-type HEK-293 cell lysate

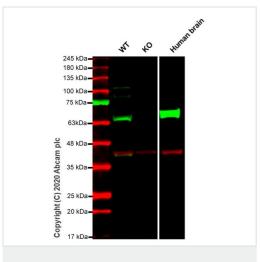
Lane 2: FYN knockout HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa Observed band size: 60 kDa

False colour image of Western blot: Anti-Fyn antibody [EPR19636] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab184276 was shown to bind specifically to Fyn. A band was observed at 60 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in FYN knockout cell line ab269630 (knockout cell lysate ab272440). To generate this image, wild-type and FYN knockout HEK-293 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-Fyn antibody [EPR19636] (ab184276)

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: FYN knockout HEK-293T cell lysate

Lane 3: Human brain tissue lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa **Observed band size:** 159 kDa

Lanes 1-3: Merged signal (red and green). Green - ab184276 observed at 60 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab184276 Anti-Fyn antibody [EPR19636] was shown to specifically react with Fyn in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266133 (knockout cell lysate ab257071) was used. Wild-type and Fyn knockout samples were subjected to SDS-PAGE. ab184276 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Fyn antibody [EPR19636] (ab184276)

Lane 1: Human brain lysate

Lane 2 : Ramos (human Burkitt's lymphoma cell line) whole cell

 $\textbf{Lane 3:} \ \textbf{HEK-293} \ (\textbf{human epithelial cell line from embryonic}$

kidney) whole cell lysate

Lane 4: P0 mouse brain lysate

Lane 5: P0 rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

Lane 1 : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/4000 dilution

Lanes 2-5: Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

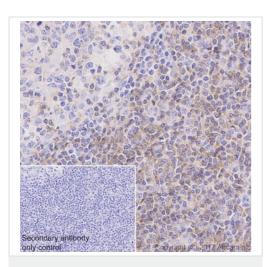
Developed using the ECL technique.

Predicted band size: 61 kDa **Observed band size:** 60 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lanes 1 and 2: 3 minutes; Lanes 3,4 and 5: 8

seconds.

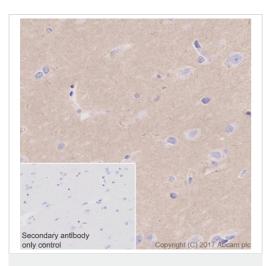


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fyn antibody
[EPR19636] (ab184276)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Fyn with ab184276 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on T cells of human tonsil (PMID: 10523617). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fyn antibody [EPR19636] (ab184276)

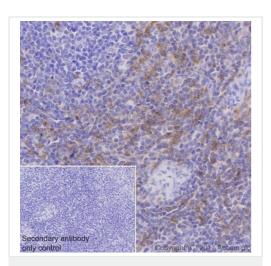
Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue labeling Fyn with ab184276 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on human cerebral cortex (PMID: 7544314).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

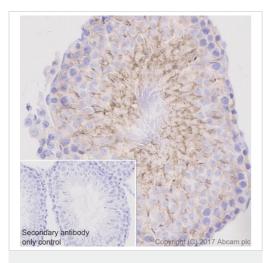


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fyn antibody
[EPR19636] (ab184276)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling Fyn with ab184276 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on T cells of mouse spleen (PMID: 7544314, PMID:10523617). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

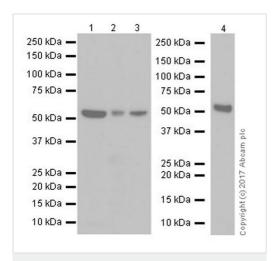


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Fyn antibody
[EPR19636] (ab184276)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling Fyn with ab184276 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous staining on rat testis (PMID: 7544314). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Fyn antibody [EPR19636] (ab184276)

Lane 1 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 2: PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 3: NIH/3T3 (mouse embyro fibroblast cell line) whole cell lysate

Lane 4: Rat spleen lysate

Lysates/proteins at 10 µg per lane.

Secondary

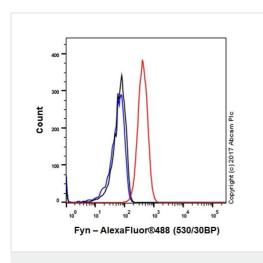
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 61 kDa **Observed band size:** 60 kDa

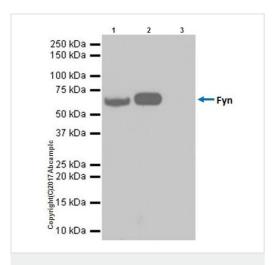
Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-Fyn antibody [EPR19636] (ab184276)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cell line labeling Fyn with ab184276 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Fyn antibody [EPR19636] (ab184276)

Fyn was immunoprecipitated from 0.35 mg of P0 mouse brain lysate with ab184276 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab184276 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: P0 mouse brain lysate 10 µg (Input).

Lane 2: ab184276 IP in P0 mouse brain lysate.

Lane 3: Rabbit monoclonal $\lg G (\underline{ab172730})$ instead of ab184276 in P0 mouse brain lysate.

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

Exposure time: 3 minutes.



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