abcam

Product datasheet

Anti-Phosphotyrosine antibody [EPR16871] ab179530

Recombinant RabMAb

15 References 11 Images

Overview

Product name	Anti-Phosphotyrosine antibody [EPR16871]
Description	Rabbit monoclonal [EPR16871] to Phosphotyrosine
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP, ELISA, Dot blot
Species reactivity	**Reacts with:** Species independent
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Whole cell lysate from A431, L6 and NIH/3T3 cells treated with pervanadate. ICC/IF: C2C12 cells treated with Hydrogen peroxide. Flow Cyt (intra): A431 cells treated with pervanadate. IP: Whole cell extract from A431 cells treated with pervanadate.
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	Liquid
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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16871
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab179530 in the following tested applications.

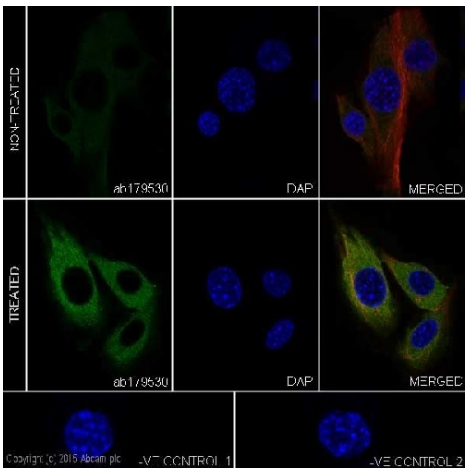
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/160. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000.
ICC/IF		1/100.
IP		1/100.
ELISA		1/6400.
Dot blot		1/1000.

Target

Relevance The phosphorylation of specific tyrosine residues has been shown to be a primary mechanism of signal transduction during normal mitogenesis, cell cycle progression and oncogenic transformation, its role in other areas such as differentiation and gap junction communication, is a matter of active and ongoing research. Antibodies that specifically recognize phosphorylated tyrosine residues have proved to be invaluable to the study of tyrosine phosphorylated proteins and the biochemical pathways in which they function.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

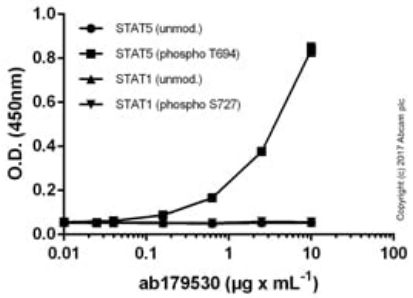
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C2C12 (Mouse myoblast cell line) cells labeling Phosphotyrosine with ab179530 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Cytoplasm staining on C2C12 cells is observed. The expression increased after treatment with H2O2 (2mM) for 10 minutes. The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control 1: - ab179530 at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.

-ve control 2: - **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG

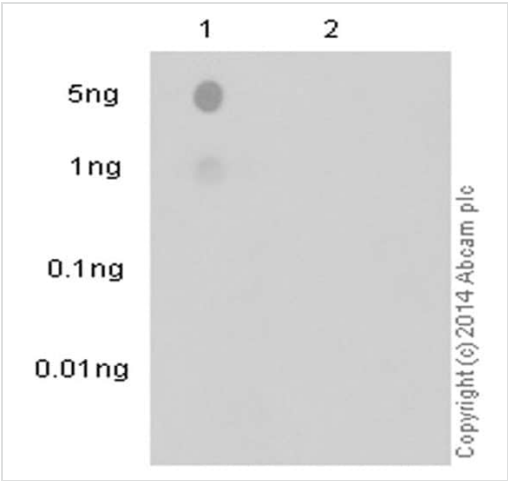
H&L) at 1/400 dilution.



ELISA - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

Serially diluted ab179530 was bound to immobilised phospho- or control peptides (STAT1 (phospho S727), STAT1 control, STAT5 (phospho T694), STAT5 control); 1 microgram per mL).

The antibody was detected by Goat anti-Rabbit HRPO and signal was developed by TMB substrate.

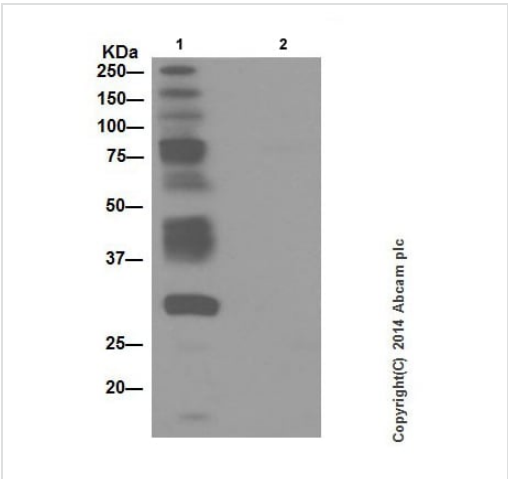


Dot Blot - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

Dot blot analysis of INSR/IGF-1R (pY1009) phospho peptide (lane 1) and INSR/IGF-1R non-phospho peptide (lane 2) labelling Phosphotyrosine with ab179530 at a dilution of 1/1000. A peroxidase-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/2500).

Blocking and dilution buffer: 5% NFDM/TBST.

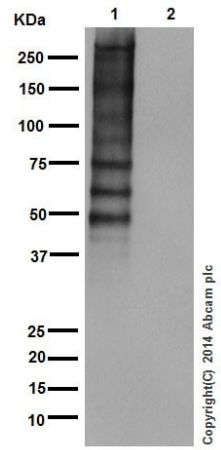
Exposure time: 3 minutes.



Immunoprecipitation - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

Phosphotyrosine was immunoprecipitated from 1mg of A431 (Human epidermoid carcinoma) whole cell extract treated with 1mM pervanadate for 30 minutes with ab179530 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab179530 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution. Lane 1: A431 treated with 1mM pervanadate for 30 minutes whole cell extract. Lane 2: PBS instead of A431 whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Multiple bands represent phosph-tyrosine containing proteins precipitated and detected by ab179530.



Western blot - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

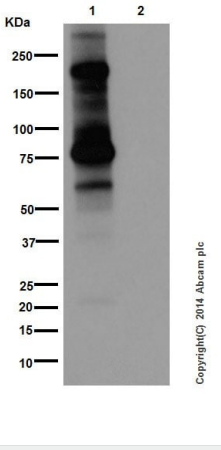
All lanes : Anti-Phosphotyrosine antibody [EPR16871] (ab179530) at 1/1000 dilution

Lane 1 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates treated with 1mM pervanadate for 20minutes
Lane 2 : Untreated Jurkat whole cell lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (H+L) Peroxidase conjugated at 1/1000 dilution

Multiple bands represent phosph-tyrosine containing proteins detected by ab179530



Western blot - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

All lanes : Anti-Phosphotyrosine antibody [EPR16871] (ab179530) at 1/10000 dilution

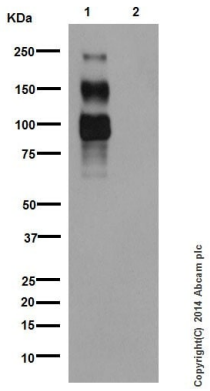
Lane 1 : A431 (Human epidermoid carcinoma) whole cell lysates treated with 50mM pervanadate for 30 minutes
Lane 2 : Untreated A431 whole cell lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Multiple bands represent phosph-tyrosine containing proteins detected by ab179530.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

All lanes : Anti-Phosphotyrosine antibody [EPR16871] (ab179530) at 1/1000 dilution

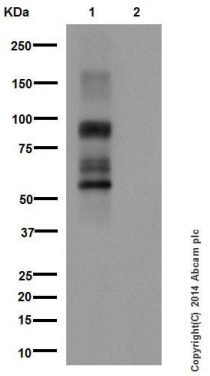
Lane 1 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysates treated with 1mM pervanadate for 10 minutes
Lane 2 : Untreated NIH/3T3 whole cell lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Multiple bands represent phosph-tyrosine containing proteins detected by ab179530.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

All lanes : Anti-Phosphotyrosine antibody [EPR16871] (ab179530) at 1/1000 dilution

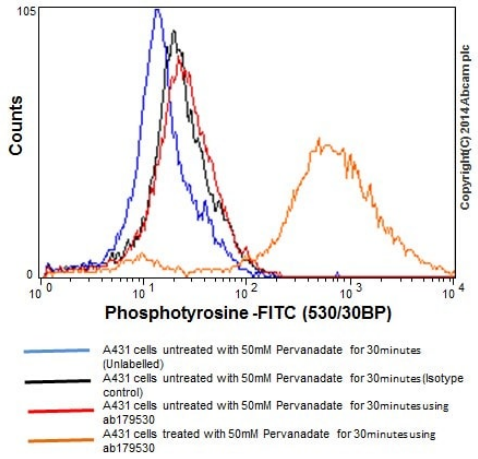
Lane 1 : L6 (Rat skeletal muscle cell line) whole cell lysates treated with 1mM pervanadate for 20 minutes
Lane 2 : Untreated L6 whole cell lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

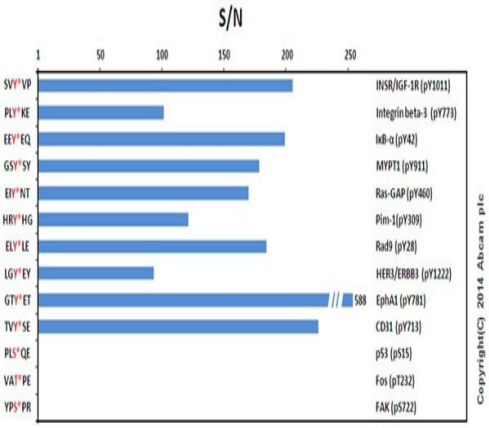
Multiple bands represent phosph-tyrosine containing proteins detected by ab179530.

Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

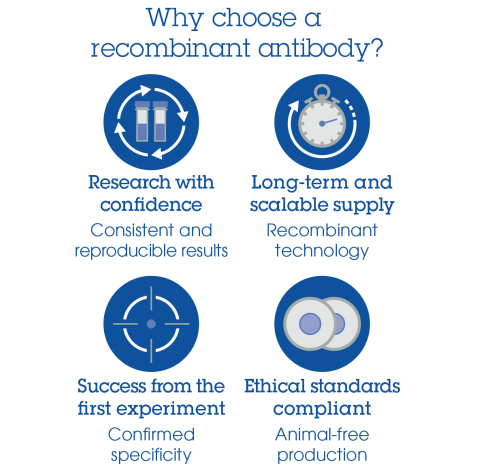
Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed Pervanadate (50mM, 30min.) treated (orange)/untreated (red)A431 (Human epidermoid carcinoma) cells labeling Phosphotyrosine with ab179530 at 1/160 dilutioncompared with a rabbit monoclonal IgG control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



ELISA - Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

ELISA analysis of various antigens (1 µg/ml) using ab179530 at 1/6400 dilution followed by Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution.

S/N = signal-to-noise ratio of phospho- versus nonphospho-peptides.



Anti-Phosphotyrosine antibody [EPR16871] (ab179530)

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