# abcam

# Product datasheet

# Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free ab215978





★★★★★ 1 Abreviews 11 Images

#### Overview

**Product name** Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free

**Description** Rabbit monoclonal [Y92] to PDGFR alpha + PDGFR beta - Low endotoxin, Azide free

**Host species** Rabbit

Specificity Expression levels of the target protein vary with sample type and some optimisation may be

required.

**Tested applications** Suitable for: ICC/IF, Flow Cyt (Intra), WB, ELISA, IP, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: N-GST tagged Human PDGF Receptor beta (aa557 to 1106) recombinant protein, N-GST

> tagged Human PDGF Receptor alpha (aa550 to 1089) recombinant protein, NIH/3T3 cell lysate. SH-SY5Y cell lysate. Rat brain and heart tissue lysate. Mouse brain tissue lysate. Human fetal brain tissue lysate. Human skeletal muscle tissue lysate. ICC/IF: NIH/3T3 cells. IHC-P: Human prostatic carcinoma, lung cancer, breast and spleen tissue. Flow Cyt (intra): NIH/3T3 cells. IP:

NIH/3T3 cell lysate.

**General notes** ab215978 is the carrier-free version of ab32570.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

Our <u>Low endotoxin, azide-free formats</u> have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

## **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Storage buffer** pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

**Clonality** Monoclonal

Clone number Y92 Isotype IgG

## **Applications**

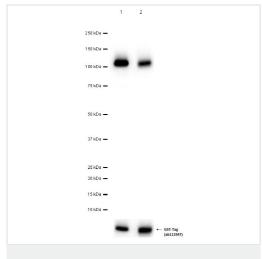
The Abpromise guarantee Our Abpromise guarantee covers the use of ab215978 in the following tested applications.

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

Application	Abreviews	Notes
ICC/IF	**** <u>(1)</u>	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.  Primary antibody concentration range: 1000 - 0 ng/mL
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

#### **Target**

#### **Images**



Western blot - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978) **All lanes :** Anti-PDGFR alpha + PDGFR beta antibody [Y92] - C-terminal (ab32570) at 1/1000 dilution

Lane 1 : Recombinant human PDGFR beta protein (ab60833)

Lane 2: Recombinant human PDGFR alpha protein (ab84797)

Lysates/proteins at 0.1 µg per lane.

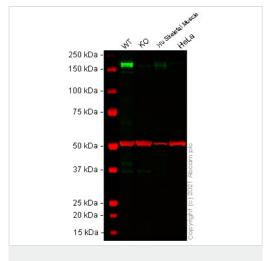
# **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).

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

Exposure time: 3 seconds



Western blot - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978) **All lanes :** Anti-PDGFR alpha + PDGFR beta antibody [Y92] - C-terminal (**ab32570**) at 1/5000 dilution

Lane 1: Wild-type SH-SY5Y cell lysate

Lane 2: PDGFR beta knockout SH-SY5Y cell lysate

Lane 3: Human Skeletal Muscle tissue lysate

Lane 4: HeLa cell lysate

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

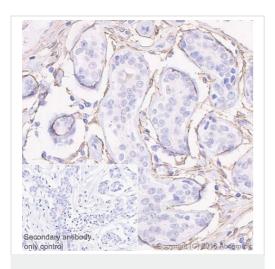
Observed band size: 170 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab32570</u>).

Lanes 1 - 4: Merged signal (red and green). Green - ab32570

observed at 170 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

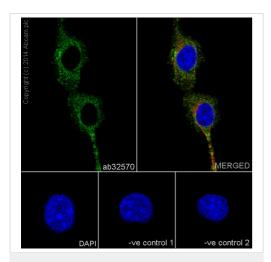
ab32570 was shown to react with PDGFRB in wild-type SH-SY5Y cells in Western blot with loss of signal observed in PDGFRB knockout cell line ab273749 (knockout cell lysate ab275523). Wild-type SH-SY5Y and PDGFRB knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab32570 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



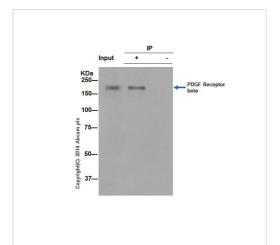
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

<u>ab32570</u> staining PDGFR alpha + beta in human breast tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Shows positive staining on stromal cells. Heat mediated antigen retrieval was performed using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody (1/500). An HRP-conjugated Goat anti-rabbit IgG (ready to use) was used as the secondary antibody. Counter stained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).



Immunocytochemistry/ Immunofluorescence - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)



Immunoprecipitation - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

Immunofluorescence staining of NIH/3T3 (Mouse embryo fibroblast cell line) cells with purified <u>ab32570</u> at a working dilution of 1 in 100, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (<u>ab7291</u>) and Alexa Fluor<sup>®</sup> 594 goat anti-mouse at a dilution of 1/500 (<u>ab150120</u>). The secondary antibody was <u>ab150077</u> Alexa Fluor<sup>®</sup> 488 goat anti rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100.

The negative controls are shown in the bottom middle and right hand panels - for the first negative control, purified <u>ab32570</u> was used at a dilution of 1/200 followed by an Alexa Fluor<sup>®</sup> 555 goat anti-mouse antibody at a dilution of 1/500 and for the second negative control mouse primary antibody (<u>ab7291</u>) and anti-rabbit secondary antibody (<u>ab15007</u>) were used.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).

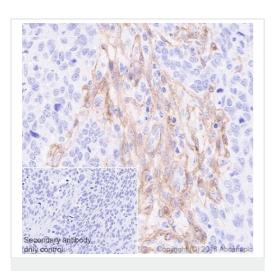
<u>ab32570</u> (purified) at 1/20 immunoprecipitating PDGFR alpha + beta in NIH/3T3 (Mouse embryo fibroblast cell line) (Lane 1 and 2). Lane 3 - PBS.

For western blotting a HRP-conjugated anti-rabbit lgG specific to the non-reduced form of lgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

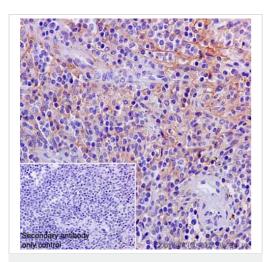
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

ab32570 staining PDGFR alpha + beta in human lung cancer tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Shows positive staining on stromal cells. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody (1/500). An HRP-conjugated Goat anti-rabbit IgG (ready to use) was used as the secondary antibody. Counter stained with Hematoxylin.

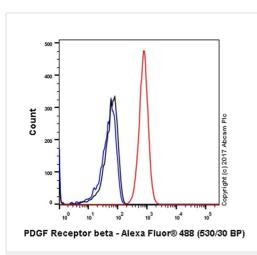
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

Immunohistochemical staining of paraffin embedded human spleen with purified <u>ab32570</u> at a working dilution of 1/50. The secondary antibody used is <u>ab97051</u>, a HRP-conjugated goat anti-rabbit lgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

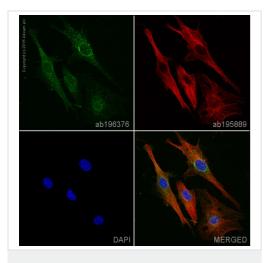
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).



Flow Cytometry (Intracellular) - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

Intracellular Flow Cytometry analysis of NIH/3T3 (Mouse embryo fibroblast cell line) cells labeling PDGFR alpha +beta (red) with **ab32570** at a 1/20 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor<sup>®</sup> 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (**ab172730**). Blue (unlabeled control) - Cells without incubation with primary and secondary antibodies.

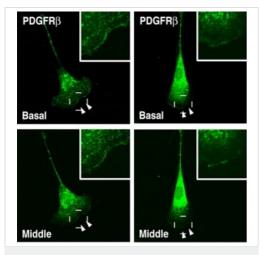
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32570).



Immunocytochemistry/ Immunofluorescence - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

Clone Y92 (ab215978) has been successfully conjugated by Abcam. This image was generated using Anti-PDGFR beta antibody [Y92] (Alexa Fluor® 488). Please refer to <a href="mailto:ab196376">ab196376</a> for protocol details.

ab196376 staining PDGFR alpha + beta in NIH3T3 cells. The cells were fixed with 100% methanol (5 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab196376 at 1/50 dilution (shown in green) and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at 1/167 dilution overnight at +4°C. Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-PDGFR alpha + PDGFR beta antibody [Y92] - Low endotoxin, Azide free (ab215978)

Image from Myata Met al. J Biol Chem. 2009 Sep 4;284(36):24595-609. Epub 2009 Jul 9. Fig 1.; doi: 10.1074/jbc.M109.016436; September 4 2009 The Journal of Biological Chemistry 284 24595-24609.

Immunofluorescence analysis of NIH/3T3 (Mouse embryo fibroblast cell line) cells stimulated with PDGF, staining PDGFR alpha + beta with unpurified <u>ab32570</u>.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32570</u>).



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