

p47phox Polyclonal Antibody

Catalog NumberPA1-9073

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human, Mouse, Pig
Host/Isotope	Goat / IgG	Published species	Human, Mouse, Not Applicable
Class	Polyclonal	Tested Applications	
Type	Antibody	Flow Cytometry (Flow)	Dilution *10 µg/mL
Immunogen	Synthetic peptide corresponding to the C terminus amino acids SESTKRKLASAV	Immunohistochemistry (IHC)	5 µg/mL
		Western Blot (WB)	0.01-1 µg/mL
Conjugate	Unconjugated	Immunocytochemistry (ICC/IF)	10 µg/mL
Form	Liquid	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Concentration	0.5 mg/mL		
Purification	Ammonium sulfate precipitation		
Storage buffer	TBS, pH 7.3, with 0.5% BSA		
Contains	0.02% sodium azide		
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles		

Product specific information

This antibody is predicted to react with bovine, mouse, porcine, rabbit and rat based on sequence homology. This antibody is tested in Peptide ELISA: antibody detection limit dilution 20,000.

Background/Target Information

p47phox (NCF1) is required, along with NCF2 and membrane bound cytochrome b558, for activation of the latent NADPH oxidase. This oxidase is a multicomponent enzyme that is activated to produce superoxide anion. Mutations in the gene can result in granulomatous disease.

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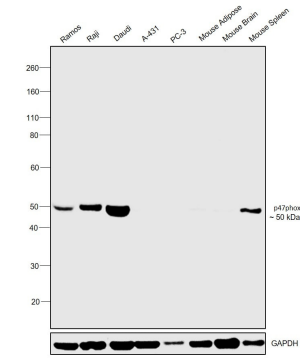
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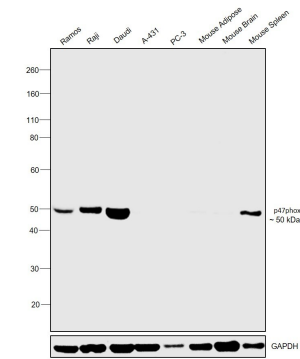
p47phox Antibody (PA1-9073)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell lysates and tissue extracts tested owing to their inherent genetic constitution. Relative expression of p47phox was observed in Ramos, Raji, Daudi and Mouse Spleen as compared to A-431, PC-3, Mouse Adipose and Mouse Brain using p47phox Polyclonal Antibody (Product # PA1-9073) in Western Blot. {RE}



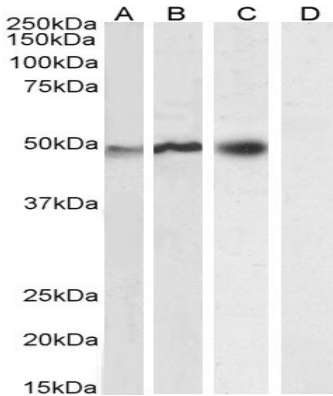
p47phox Antibody (PA1-9073) in WB

Western blot was performed using Anti-p47phox Polyclonal Antibody (Product # PA1-9073) and a 50 kDa band corresponding to p47phox was observed across cell lines and tissue extract tested except A-431, PC-3, Mouse Adipose and Mouse Brain which are reported to be low. Membrane enriched extracts (30 µg lysate) of Ramos (Lane 1), Raji (Lane 2), Daudi (Lane 3), A-431 (Lane 4) and PC-3 (Lane 5) and tissue extracts of Mouse adipose (Lane 6), Mouse Brain (Lane 7) and Mouse Spleen (Lane 8) were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (0.5 µg/mL) and detected by chemiluminescence with Rabbit anti-Goat IgG Heavy Chain, Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27014, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



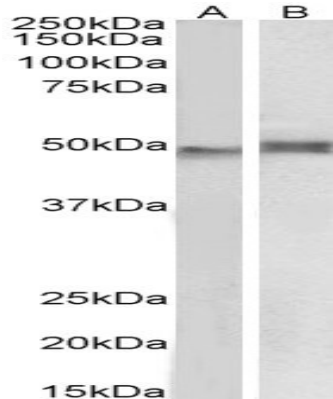
p47phox Antibody (PA1-9073) in WB

Western blot analysis of p47phox using p47phox Polyclonal Antibody (Product # PA1-9073) (0.2 µg/mL) in staining of U937 (A), (0.01 µg/mL) Daudi (B), (0.3 µg/mL) U251 (C) and negative control A431 (D) cell lysate (35 µg protein in RIPA buffer). Detected by chemiluminescence.



p47phox Antibody (PA1-9073) in WB

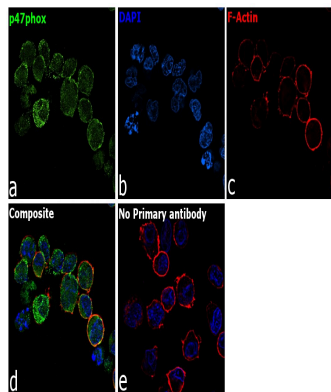
Western blot analysis of p47phox using p47phox Polyclonal Antibody (Product # PA1-9073) (1 µg/mL) in staining of Mouse Thymus (A) and Pig Spleen (B) lysate (35 µg protein in RIPA buffer). Detected by chemiluminescence.



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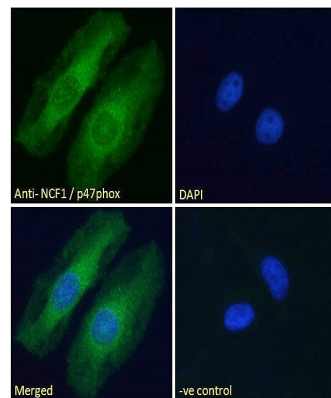
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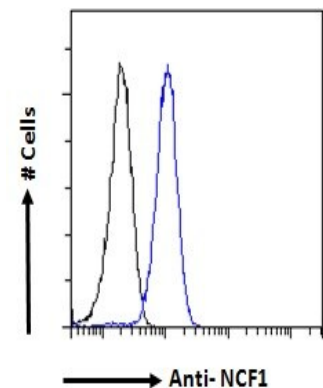
p47phox Antibody (PA1-9073) in ICC/IF

Immunofluorescence analysis of p47phox was performed using 70% confluent log phase Raji cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with p47phox Goat Polyclonal Antibody (Product # PA1-9073) at 5 µg/mL in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Rabbit anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Product # A-11078) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415). Panel d represents the merged image showing cytoplasmic and membrane localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



p47phox Antibody (PA1-9073) in ICC/IF

Immunocytochemical analysis of p47phox in HeLa cells using a p47phox polyclonal antibody (Product #PA1-9073). HeLa cells were permeabilized with 0.15% Triton. Cells were incubated with 10 µg/mL of primary antibody for one hour followed by an Alexa Fluor 488 secondary antibody at a concentration of 2 µg/mL. Cytoplasmic staining can be seen as shown above. The nuclear stain is DAPI (blue). Negative control: Unimmunized goat IgG (10 µg/mL) followed by an Alexa Fluor 488 secondary antibody (2 µg/mL).



p47phox Antibody (PA1-9073) in Flow

Flow cytometric analysis of p47phox in HeLa cells using a polyclonal antibody (Product #PA1-9073). HeLa cells (blue line) were paraformaldehyde fixed and permeabilized with 0.5% Triton. The primary antibody was incubated for one hour (10 µg/mL) followed by an Alexa Fluor 488 secondary antibody (1 µg/mL). IgG control: Unimmunized goat IgG (black line) followed by an Alexa Fluor 488 secondary antibody.

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