

p-p40-phox (B-8): sc-377550

BACKGROUND

Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase is a multi-meric enzyme system that mediates electron transport from NADPH in the cytoplasm to molecular oxygen in the phagosome, thereby generating reactive oxidant intermediates. Upon neutrophil stimulation, NADPH-oxidase and other cytosolic elements localize to the cell membrane from the cytosol to form a complex which produces phagocytic oxygen radicals. There are a number of cytosolic proteins that are involved in NADPH-oxidase activation/deactivation, including p47-phox, p67-phox, p40-phox and the small GTP-binding protein, Rac. Activation of NADPH oxidase is accompanied by the phosphorylation of cytosolic components p40-phox, p47-phox and p67-phox. The PKC consensus phosphorylation sites Thr 154 and Ser 315 in p40-phox are phosphorylated during activation of NADPH oxidase. p40-phox can promote oxidase activation by increasing the affinity of p47-phox for NADPH-oxidase. However, p40-phox appears to downregulate oxidase function as well, by competing with an SH3 domain interaction between other essential oxidase components.

CHROMOSOMAL LOCATION

Genetic locus: NCF4 (human) mapping to 22q12.3; Ncf4 (mouse) mapping to 15 E1.

SOURCE

p-p40-phox (B-8) is a mouse monoclonal antibody raised against a short amino acid sequence containing Thr 154 phosphorylated p40-phox of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2b} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

p-p40-phox (B-8) is available conjugated to agarose (sc-377550 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-377550 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-377550 PE), fluorescein (sc-377550 FITC), Alexa Fluor® 488 (sc-377550 AF488), Alexa Fluor® 546 (sc-377550 AF546), Alexa Fluor® 594 (sc-377550 AF594) or Alexa Fluor® 647 (sc-377550 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-377550 AF680) or Alexa Fluor® 790 (sc-377550 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

Blocking peptide available for competition studies, sc-377550 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

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STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

APPLICATIONS

p-p40-phox (B-8) is recommended for detection of Threonine 154 phosphorylated p40-phox of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-p40-phox (B-8) is also recommended for detection of correspondingly phosphorylated p40-phox in additional species, including canine and porcine.

Suitable for use as control antibody for p40-phox siRNA (h): sc-36155, p40-phox siRNA (m): sc-36156, p40-phox shRNA Plasmid (h): sc-36155-SH, p40-phox shRNA Plasmid (m): sc-36156-SH, p40-phox shRNA (h) Lentiviral Particles: sc-36155-V and p40-phox shRNA (m) Lentiviral Particles: sc-36156-V.

Molecular Weight of p-p40-phox: 40 kDa.

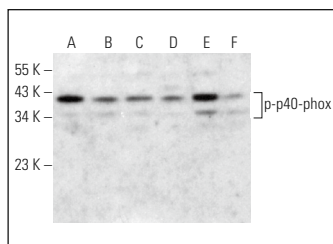
Positive Controls: CCRF-CEM cell lysate: sc-2225, RAW 264.7 whole cell lysate: sc-2211 or HL-60 whole cell lysate: sc-2209.

RECOMMENDED SUPPORT REAGENTS

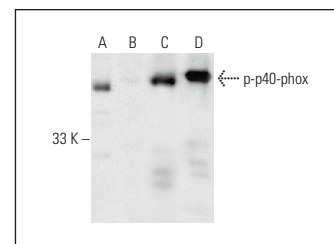
To ensure optimal results, the following support reagents are recommended:

- 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A and Western Blotting Luminol Reagent: sc-2048.
- 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).
- 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

DATA



p-p40-phox (B-8): sc-377550. Western blot analysis of p40-phox phosphorylation in CCRF-CEM (A), RAW 264.7 (B), HL-60 (C), Jurkat (D), HEL 92.1.7 (E) and M1 (F) whole cell lysates.



Western blot analysis of p40-phox phosphorylation in untreated (A, C) and lambda protein phosphatase (sc-200312A) treated (B, D) THP-1 whole cell lysates. Antibodies tested include p-p40-phox (B-8): sc-377550 (A, B) and p40-phox (B-1): sc-48376 (C, D).

SELECT PRODUCT CITATIONS

1. Hedl, M., et al. 2019. IRF5 is required for bacterial clearance in human M1-polarized macrophages, and IRF5 immune-mediated disease risk variants modulate this outcome. J. Immunol. 202: 920-930.

RESEARCH USE

For research use only, not for use in diagnostic procedures.