Product Datasheet

5-Lipoxygenase Antibody - BSA Free
NB110-58748

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Reviews: 2 Publications: 2

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
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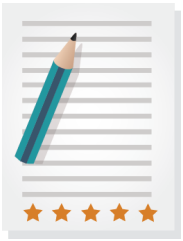
Updated 12/20/2023 v.20.1



NB110-58748

5-Lipoxygenase Antibody - BSA Free

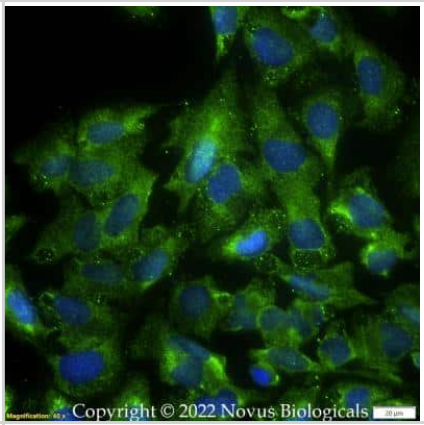
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	80 kDa

Product Description	
Host	Rabbit
Gene ID	240
Gene Symbol	ALOX5
Species	Human, Mouse
Immunogen	A synthetic peptide made to a region within residues 100-200 of human 5-Lipoxygenase. [Swiss-Prot# P09917]

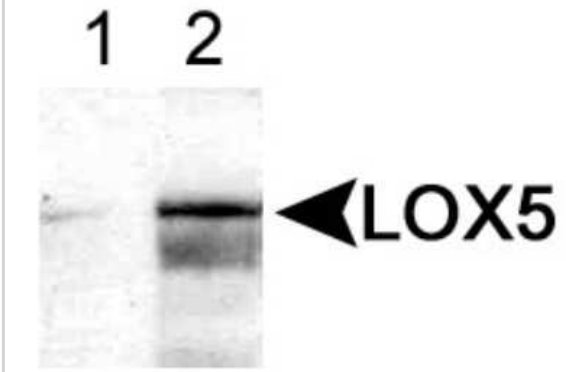
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Knockdown Validated
Recommended Dilutions	Western Blot 1:500-1:1000, Simple Western 50 ug/ml, Immunocytochemistry/ Immunofluorescence 1:500 - 1:2000, Knockdown Validated
Application Notes	This 5 Lipoxygenase antibody is useful for Immunocytochemistry/Immunofluorescence and Western Blot, where a band is seen at approx. 80 kDa. There may be some non-specific bands at non-interfering lower molecular weights. In ICC/IF cytoplasmic staining was observed in Hela cells. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Immunocytochemistry/Immunofluorescence: 5-Lipoxygenase Antibody - BSA Free [NB110-58748] - U2OS cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with 5-Lipoxygenase Antibody (NB110-58748) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

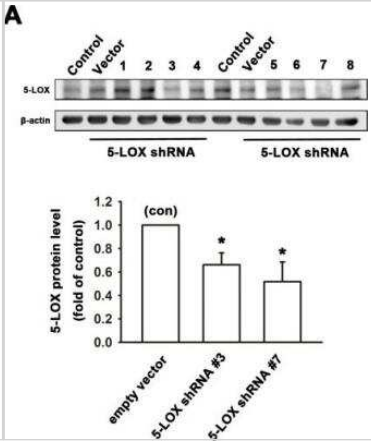


Western Blot: 5-Lipoxygenase Antibody [NB110-58748] - Detection of LOX5 in human peripheral leukocytes using NB110-58748 at 0.5(Lane 1) and 2.0 ug/ml (Lane 2).

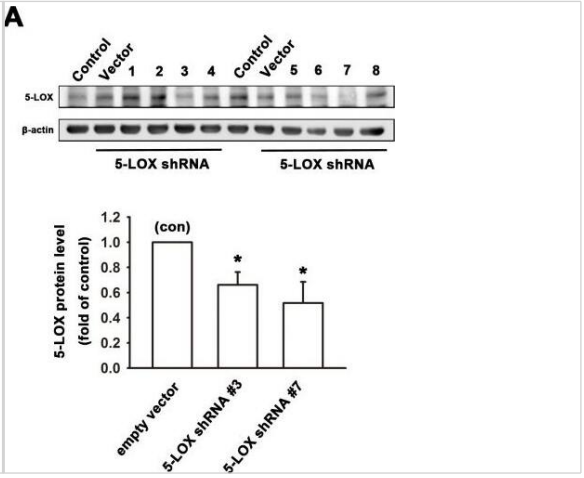


Simple Western: 5-Lipoxygenase Antibody [NB110-58748] - Simple Western lane view shows a specific band for 5-Lipoxygenase in 0.5 mg/ml of Human Lung lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. * Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody

Knockdown Validated: 5-Lipoxygenase Antibody [NB110-58748] - Knockdown of 5-Lipoxygenase inhibits TNF-alpha-induced IL-6 and MCP-1 release in human synovial fibroblasts.Total protein extracts from RASFs transfected with empty vector or 5-Lipoxygenase shRNA were used for Western blotting. Immunoblotting showed that cells transfected with 5-Lipoxygenase shRNA of clone No.3 and No.7 markedly decreased 5-Lipoxygenase expression. Human synovial fibroblasts transfected with empty vector or 5-Lipoxygenase shRNA were treated with TNF-alpha for 6 hr. IL-6 and MCP-1 protein release in the conditioned medium was evaluated by ELISA. Note that knockdown of 5-Lipoxygenase decreased IL-6. Image collected and cropped by CiteAb from the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4168259/) licensed under a CC-BY license.



Knockdown of 5-LOX inhibits TNF-alpha-induced IL-6 and MCP-1 release in human synovial fibroblasts.Total protein extracts from RASFs transfected with empty vector or 5-LOX shRNA were used for Western blotting. (A) Immunoblotting showed that cells transfected with 5-LOX shRNA of clone No.3 and No.7 markedly decreased 5-LOX expression. Human synovial fibroblasts transfected with empty vector or 5-LOX shRNA were treated with TNF-alpha for 6 hr. IL-6 and MCP-1 protein release in the conditioned medium was evaluated by ELISA. Note that knockdown of 5-LOX decreased IL-6 (B) and MCP-1 (C) release following TNF-alpha treatment for 6 hr. Data are presented as mean +/- SEM. *, p<0.05 compared with empty vector control (con). Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0107890), licensed under a CC-BY licence.



Publications

Simard M, Rioux G, Morin S et al. Investigation of Omega-3 Polyunsaturated Fatty Acid Biological Activity in a Tissue-Engineered Skin Model Involving Psoriatic Cells The Journal of investigative dermatology 2021-04-20 [PMID: 33857488]

Lin HC, Lin TH, Wu MY et al. 5-Lipoxygenase Inhibitors Attenuate TNF-a-Induced Inflammation in Human Synovial Fibroblasts. PLoS ONE. 2014-09-18 [PMID: 25229347]

Procedures

Western Blot Protocol specific for 5 Lipoxygenase Antibody (NB110-58748)
5-Lipoxygenase Antibody:
Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 38 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-LOX5 primary antibody (NB 110-58748) in blocking buffer and incubate 2 hours at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce ECL).

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence protocol for 5-Lipoxygenase Antibody (NB110-58748)
5-Lipoxygenase Antibody:
Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NB110-58748

NB820-59174	Blood Leukocyte Whole Cell Lysate
NB110-58748PEP	5-Lipoxygenase Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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