

# 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.

[www.novusbio.com](http://www.novusbio.com)



[technical@novusbio.com](mailto:technical@novusbio.com)

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**NB110-58748****5-Lipoxygenase Antibody - BSA Free**

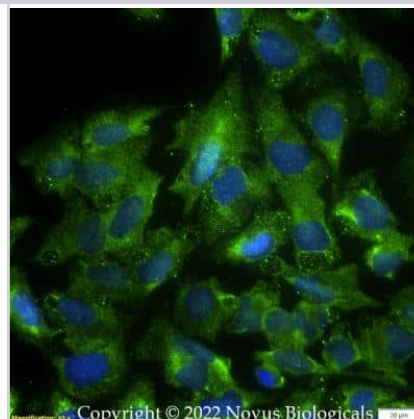
<b>Product Information</b>	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1 mg/ml
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.1% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	PBS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

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

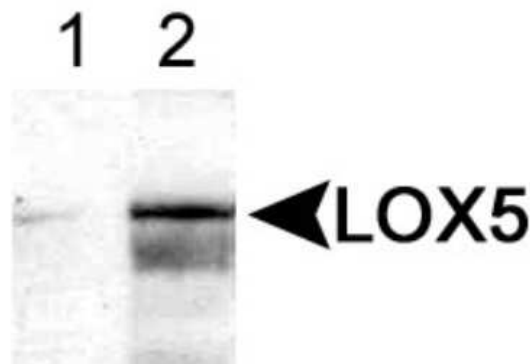
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Knockdown Validated
<b>Recommended Dilutions</b>	Western Blot 1:500-1:1000, Simple Western 50 ug/ml, Immunocytochemistry/ Immunofluorescence 1:500 - 1:2000, Knockdown Validated
<b>Application Notes</b>	<p>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.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</p> <p>See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in Human Lung lysate 0.5 mg/mL, separated by Size, antibody dilution of 50 ug/mL. Separated by Size-Wes, Sally Sue/Peggy Sue.</p> <p>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.</p>

## Images

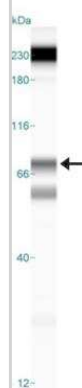
Immunocytochemistry/Immunofluorescence: 5-Lipoxygenase Antibody - BSA Free [NB110-58748] - U2OS cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with 5-Lipoxygenase Antibody (NB110-58748) at 1ug/ml overnight 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.



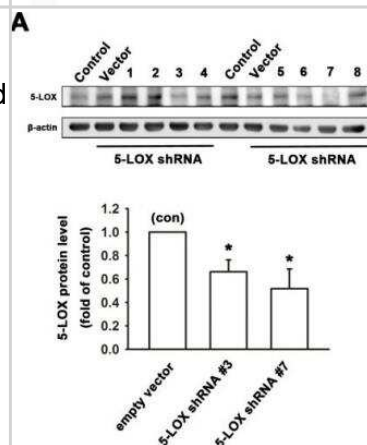
Western Blot: 5-Lipoxygenase Antibody [NB110-58748] - Detection of LOX5 in human peripheral leukocytes using NB110-58748 at 0.5(Lane 1) and 2.0 ug/ml (Lane 2).



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



Western Blot: 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.



## Publications

Marques RM, Gonzalez-Nunez M, Walker ME et al. Loss of 15-lipoxygenase disrupts T(reg) differentiation altering their pro-resolving functions *Cell Death & Differentiation* 2021-11-01 [PMID: 34040168] (In vivo assay)

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- $\alpha$ -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 dH<sub>2</sub>O 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 dH<sub>2</sub>O 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 dH<sub>2</sub>O 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 1:25,000 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.



### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NB110-58748**

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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

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### **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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