

# Product Datasheet

## Lipase A Antibody - BSA Free

### NBP1-54155SS

Unit Size: 0.025 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP1-54155SS**

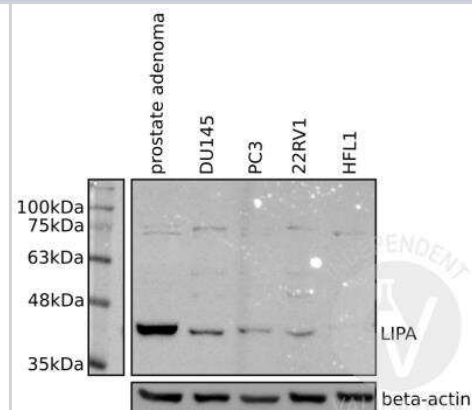
Lipase A Antibody - BSA Free

Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Product Description	
Host	Rabbit
Gene ID	3988
Gene Symbol	LIPA
Species	Human, Mouse
Reactivity Notes	Immunogen sequence has 82% identity to pig and 73% identity to rat and cow.
Immunogen	Partial recombinant protein made to an internal region of the human Lipase A protein (within residues 150-300). [Swiss-Prot P38571]
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Simple Western 1:10000, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100 - 1:500, Immunohistochemistry-Paraffin 1:100
Application Notes	<p>This LIPA antibody is useful for ICC/IF, IHC-P and Western blot, where a band is seen ~45 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</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 HepG2 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:10,000, apparent MW was 75 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

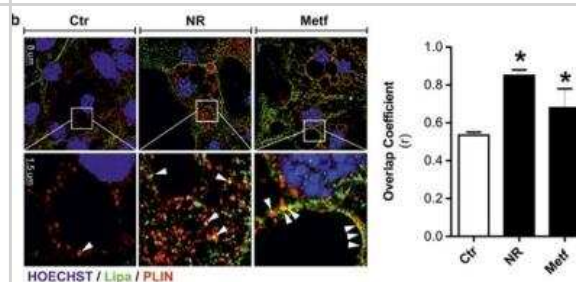


## Images

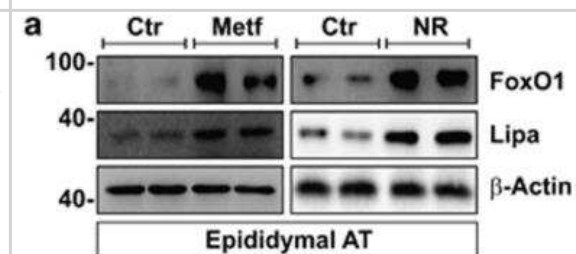
**Western Blot: Lipase A Antibody [NBP1-54155] - Analysis using NBP1-54155 on cellular extracts of human prostate adenoma tissue, DU145, PC3, and 22RV1 prostate cancer cell lines, and HFL1 Normal primary human fibroblasts.** NBP1-54155 revealed a strong specific band at the expected molecular weight for Lipase A at 45 kDa in different lysates of human cancer cell lines. NBP1-54155 specifically reveals LIPA in extracts from human prostate adenoma tissue and prostate cancer cell lines DU145, PC3, and 22RV1. The protein bands were visible in the positive but not in the negative controls. Therefore, NBP1-54155 was found to be suitable for checking the expression levels of Lipase A protein. Image from verified customer review.



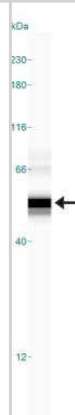
**Immunocytochemistry/Immunofluorescence: Lipase A Antibody [NBP1-54155] - Metabolic stress triggers lipophagy in adipocytes.** 3T3-L1 adipocytes were subjected to NR or treated with Metf for 8 h. Cells were immunostained with Lipa (green) and PLIN (red) antibodies. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/cddis2013404>) licensed under a CC-BY license.



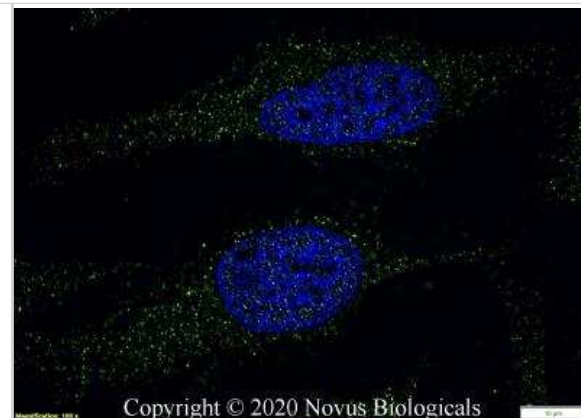
**Western Blot: Lipase A Antibody [NBP1-54155] - NR and Metf promote FoxO1-mediated Lipa upregulation in visceral AT of adult mice.** Adult C57/BL6 mice (5 months) were nutrient restricted (NR) by 24 h fasting or treated for 10 days with Metf (400 mg/kg) dissolved in drinking water (n=4 mice per group). Western blot of FoxO1 and Lipa in total protein extracts of explanted visceral (epididymal) AT. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/cddis2013404>) licensed under a CC-BY license.



**Simple Western: Lipase A Antibody [NBP1-54155] - Image shows a specific band for Lipase A in 0.5 mg/mL of HepG2 lysate.** This experiment was performed under reducing conditions using the 12-230 kDa separation system.



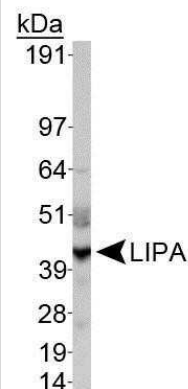
**Immunocytochemistry/Immunofluorescence: Lipase A Antibody [NBP1-54155]** - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Lipase A Antibody NBP1-54155 at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



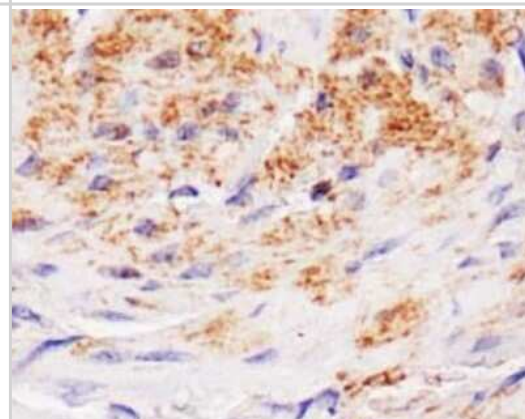
**Immunohistochemistry-Paraffin: Lipase A Antibody - BSA Free [NBP1-54155]** - Analysis of a FFPE tissue section of human stomach using 1:200 dilution of Lipase A antibody (NBP1-54155). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



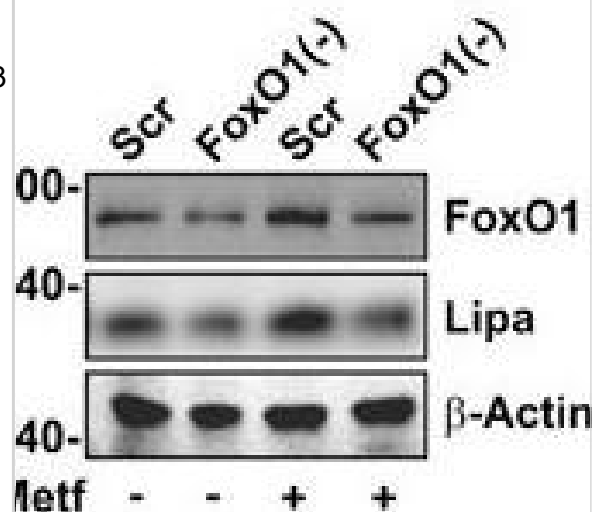
**Western Blot: Lipase A Antibody [NBP1-54155]** - Detection of LIPA in HepG2 whole cell extract.



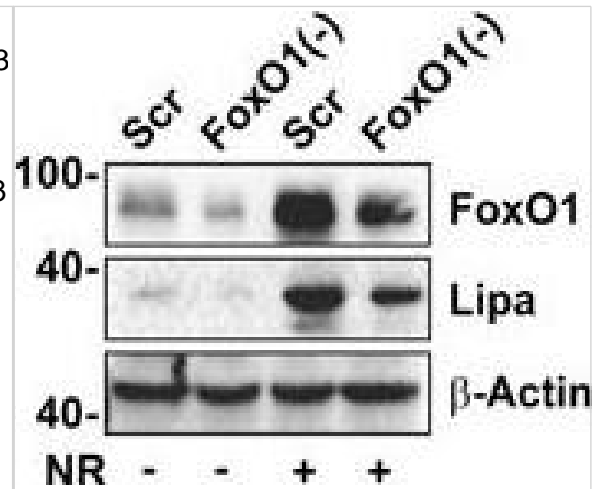
**Immunohistochemistry: Lipase A Antibody [NBP1-54155]** - Staining of LIPA in mouse stomach smooth muscle.



Western Blot: Lipase A Antibody - BSA Free [NBP1-54155] - FoxO1-mediated lysosomal acid lipase (Lipa) induction in NR & Metf-treated 3T3-L1 adipocytes. (a) WB of FoxO1, ATGL & Lipa in total protein extracts from 3T3-L1 adipocytes at different times of NR. (b) RT-qPCR analysis of relative Lipa & ATGL mRNA levels in 3T3-L1 after 4 h from NR. Dashed line indicates mRNA value of controls. (c) After 4 h from NR, 3T3-L1 adipocytes refed w/ complete cell culture medium (CM) up to 8 h. Total protein extracts used for WB analysis of FoxO1 & Lipa. (d) WB of FoxO1 in total & nuclear protein extracts from 3T3-L1 adipocytes at different times of NR. (e) ChIP assay carried out on crosslinked nuclei from 3T3-L1 adipocytes subjected to NR for 4 h & Metf for 16 h by using FoxO1 antibody followed by qPCR analysis of FoxO1RE on Lipa promoter (-51 bp). Dashed line indicates IgG value. (f & g) 3T3-L1 adipocytes transfected w/ siRNA against FoxO1 (FoxO1(-)) or w/ a scramble siRNA (Scr). WB of FoxO1 & Lipa (f) & RT-qPCR analysis of relative Lipa mRNA level (g) performed in 3T3-L1 adipocytes 4 h after NR. (h) WB of FoxO1 & Lipa in 3T3-L1 adipocytes at different times of 5 mM Metformin (Metf) treatment. (i) Confocal analysis of FoxO1 localization in 3T3-L1 adipocytes treated w/ 5 mM Metf for 16 h. Nuclei stained w/ Hoechst 33342. Colocalization plugin (ImageJ Software) used to identify FoxO1-Hoechst colocalization (white spots). (j) RT-qPCR analysis of relative Lipa mRNA level performed in 3T3-L1 adipocytes treated w/ Metf for 16 h. (k) 3T3-L1 adipocytes transfected w/ siRNA against FoxO1 (FoxO1(-)) or w/ a scramble siRNA (Scr). WB of FoxO1 & Lipa performed in 3T3-L1 adipocytes treated w/ 5 mM Metf for 24 h. All values given as mean $\pm$ S.D. (n=4). \*P<0.05, \*\*P<0.01 versus controls. °P<0.05 versus NR Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24136225>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

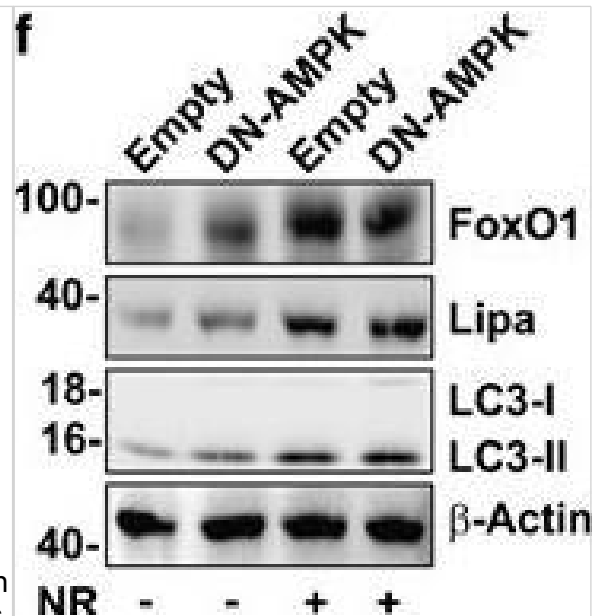


Western Blot: Lipase A Antibody - BSA Free [NBP1-54155] - FoxO1-mediated lysosomal acid lipase (Lipa) induction in NR & Metf-treated 3T3-L1 adipocytes. (a) WB of FoxO1, ATGL & Lipa in total protein extracts from 3T3-L1 adipocytes at different times of NR. (b) RT-qPCR analysis of relative Lipa & ATGL mRNA levels in 3T3-L1 after 4 h from NR. Dashed line indicates mRNA value of controls. (c) After 4 h from NR, 3T3-L1 adipocytes refed w/ complete cell culture medium (CM) up to 8 h. Total protein extracts used for WB analysis of FoxO1 & Lipa. (d) WB of FoxO1 in total & nuclear protein extracts from 3T3-L1 adipocytes at different times of NR. (e) ChIP assay carried out on crosslinked nuclei from 3T3-L1 adipocytes subjected to NR for 4 h & Metf for 16 h by using FoxO1 antibody followed by qPCR analysis of FoxO1RE on Lipa promoter (-51 bp). Dashed line indicates IgG value. (f & g) 3T3-L1 adipocytes transfected w/ siRNA against FoxO1 (FoxO1(-)) or w/ a scramble siRNA (Scr). WB of FoxO1 & Lipa (f) & RT-qPCR analysis of relative Lipa mRNA level (g) performed in 3T3-L1 adipocytes 4 h after NR. (h) WB of FoxO1 & Lipa in 3T3-L1 adipocytes at different times of 5 mM Metformin (Metf) treatment. (i) Confocal analysis of FoxO1 localization in 3T3-L1 adipocytes treated w/ 5 mM Metf for 16 h. Nuclei stained w/ Hoechst 33342. Colocalization plugin (ImageJ Software) used to identify FoxO1-Hoechst colocalization (white spots). (j) RT-qPCR analysis of relative Lipa mRNA level performed in 3T3-L1 adipocytes treated w/ Metf for 16 h. (k) 3T3-L1 adipocytes transfected w/ siRNA against FoxO1 (FoxO1(-)) or w/ a scramble siRNA (Scr). WB of FoxO1 & Lipa performed in 3T3-L1 adipocytes treated w/ 5 mM Metf for 24 h. All values given as mean $\pm$ S.D. (n=4). \*P<0.05, \*\*P<0.01 versus controls. °P<0.05 versus NR Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24136225>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

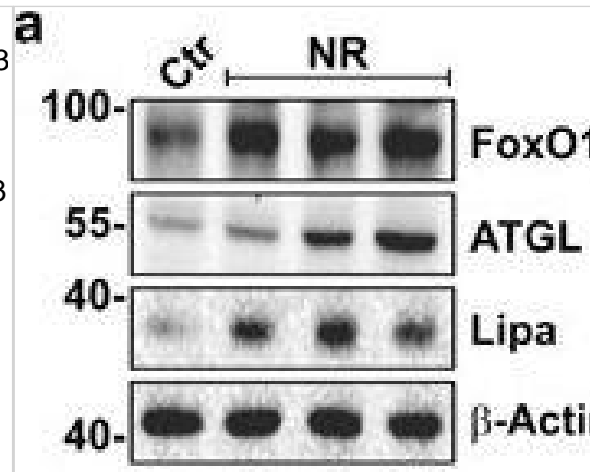




Western Blot: Lipase A Antibody - BSA Free [NBP1-54155] - AMPK drives Lipa-released FFAs oxidation restraining energetic catastrophe. (a) 3T3-L1 cells were transfected with DN-AMPK or empty vector. RT-qPCR analysis of relative peroxisome proliferator-activated receptor gamma-1 $\alpha$ , peroxisome proliferator-activated receptor- $\alpha$ , carnitine palmitoyltransferase 1b & acyl-CoA oxidase 1 mRNA levels were performed after 4 h of NR or 16 h of Metf treatment. Dashed line indicates the mRNA value of untreated DN-AMPK cells (Ctr). mRNA levels of untreated cells transfected with empty vector were similar to untreated DN-AMPK cells (data not shown). (b) Chemiluminescent assay of ATP level in 3T3-L1 adipocytes transfected with DN-AMPK or empty vector after 8 h NR or 16 h Metf treatment. ATP level was expressed as pmol ATP per mg protein. (c) After 8 h of NR or 16 h Metf treatment, FFAs were enzymatically detected in culture medium of 3T3-L1 adipocytes transfected with DN-AMPK or empty vector. Values were expressed as  $\mu$ g FFAs per mg protein. (d) Left panel: western blot of AMPKpT172, PARP-1 & cleaved form of caspase-3 in 3T3-L1 adipocytes transfected with DN-AMPK or empty vector & subjected to 8 h NR. Right panel: cytofluorimetric analysis of apoptosis in DN-AMPK cells subjected to 8 h NR. (e) Western blot of PARP-1 & cleaved form of caspase-3 in 3T3-L1 adipocytes transfected with DN-AMPK or empty vector & treated with Metf for 16 h. (f) Western blot of FoxO1, Lipa, LC3 in 3T3-L1 adipocytes transfected with DN-AMPK or empty vector & subjected to 4 h NR.  $\beta$ -actin was used as loading control. All values are given as mean $\pm$ S.D. \* $P$ <0.05, \*\* $P$ <0.01 versus controls; ° $P$ <0.05, °° $P$ <0.01 versus Metf treatment. All data are representative of at least three independent experiments Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24136225>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

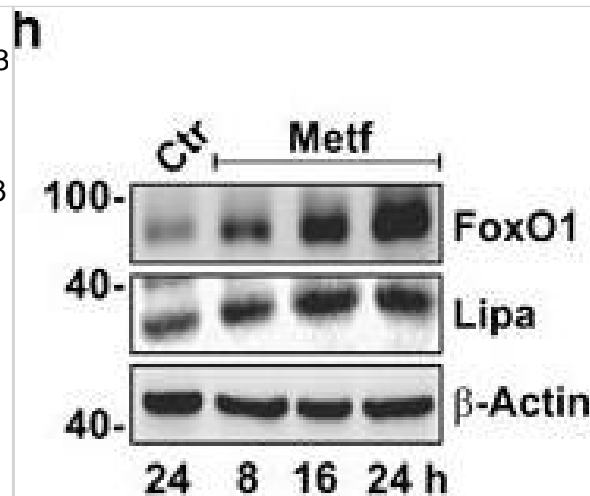


Western Blot: Lipase A Antibody - BSA Free [NBP1-54155] - FoxO1-mediated lysosomal acid lipase (Lipa) induction in NR & Metf-treated 3T3-L1 adipocytes. (a) WB of FoxO1, ATGL & Lipa in total protein extracts from 3T3-L1 adipocytes at different times of NR. (b) RT-qPCR analysis of relative Lipa & ATGL mRNA levels in 3T3-L1 after 4 h from NR. Dashed line indicates mRNA value of controls. (c) After 4 h from NR, 3T3-L1 adipocytes refed w/ complete cell culture medium (CM) up to 8 h. Total protein extracts used for WB analysis of FoxO1 & Lipa. (d) WB of FoxO1 in total & nuclear protein extracts from 3T3-L1 adipocytes at different times of NR. (e) ChIP assay carried out on crosslinked nuclei from 3T3-L1 adipocytes subjected to NR for 4 h & Metf for 16 h by using FoxO1 antibody followed by qPCR analysis of FoxO1RE on Lipa promoter (-51 bp). Dashed line indicates IgG value. (f & g) 3T3-L1 adipocytes transfected w/ siRNA against FoxO1 (FoxO1(-)) or w/ a scramble siRNA (Scr). WB of FoxO1 & Lipa (f) & RT-qPCR analysis of relative Lipa mRNA level (g) performed in 3T3-L1 adipocytes 4 h after NR. (h) WB of FoxO1 & Lipa in 3T3-L1 adipocytes at different times of 5 mM Metformin (Metf) treatment. (i) Confocal analysis of FoxO1 localization in 3T3-L1 adipocytes treated w/ 5 mM Metf for 16 h. Nuclei stained w/ Hoechst 33342. Colocalization plugin (ImageJ Software) used to identify FoxO1-Hoechst colocalization (white spots). (j) RT-qPCR analysis of relative Lipa mRNA level performed in 3T3-L1 adipocytes treated w/ Metf for 16 h. (k) 3T3-L1 adipocytes transfected w/ siRNA against FoxO1 (FoxO1(-)) or w/ a scramble siRNA (Scr). WB of FoxO1 & Lipa performed in 3T3-L1 adipocytes treated w/ 5 mM Metf for 24 h. All values given as mean $\pm$ S.D. (n=4). \*P<0.05, \*\*P<0.01 versus controls. °P<0.05 versus NR Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24136225>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

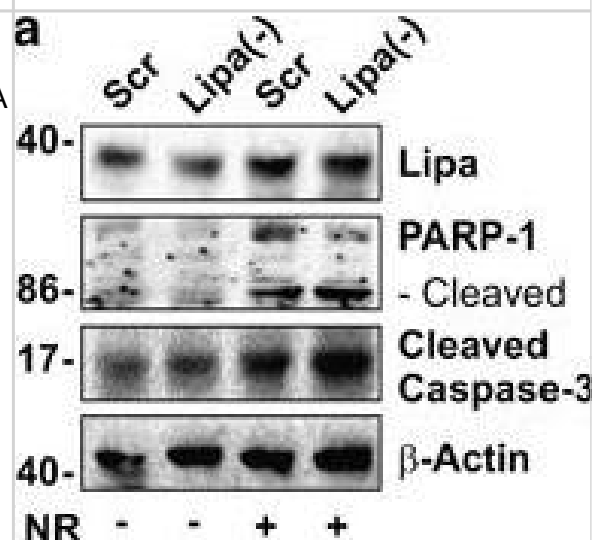




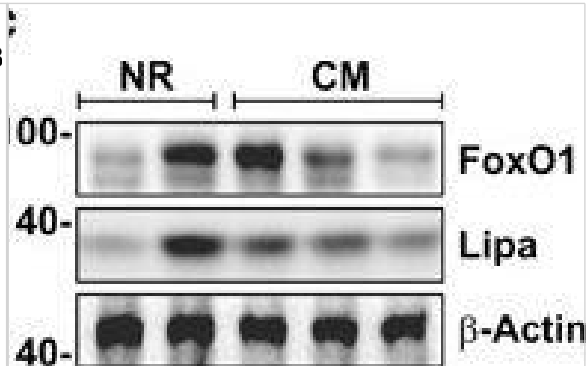
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Western Blot: Lipase A Antibody - BSA Free [NBP1-54155] - Lipa downregulation impairs lipid breakdown & elicits cell death in nutrient restricted adipocytes. (a) 3T3-L1 adipocytes were transfected with siRNA against Lipa (Lipa(-)) or with a scramble siRNA (Scr). Western blot of Lipa, PARP-1 & cleaved form of caspase-3 in total protein extracts from 3T3-L1 adipocytes after 4 h of NR. (b) TG content was quantified by ORO staining in fixed 3T3-L1 adipocytes 6 h after NR. (c) RT-qPCR analysis of relative peroxisome proliferator-activated receptor gamma-1α, peroxisome proliferator-activated receptor-α & carnitine palmitoyltransferase 1b mRNA levels was performed in 3T3-L1 adipocytes 4 h after NR. (d) FFAs were analyzed in culture medium 6 h after NR. β-actin was used as loading control. All values are given as mean±S.D. \*P<0.05, \*\*P<0.01 versus controls; °P<0.05 versus NR treatment. All data are representative of at least three independent experiments Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24136225>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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## Publications

Carotti S, Lettieri-Barbato D, Piemonte F Et al. Molecular and histological traits of reduced lysosomal acid lipase activity in the fatty liver Cell death & disease 2021-11-18 [PMID: 34795230]

Liu Z, Gomez CR, Espinoza I et al. Correlation of cholesteryl ester metabolism to pathogenesis, progression and disparities in colorectal Cancer Lipids in health and disease 2022-02-16 [PMID: 35172832] (IF/IHC, Human)

Li F, Zhao X, Li H Et al. Hepatic lysosomal acid lipase drives the autophagy-lysosomal response and alleviates cholesterol metabolic disorder in ApoE deficient mice Biochimica et biophysica acta. Molecular and cell biology of lipids 2021-08-17 [PMID: 34416392] (IF/IHC, Mouse)

Irungbam K, Roderfeld M, Glimm H et al. Cholestasis impairs hepatic lipid storage via AMPK and CREB signaling in hepatitis B virus surface protein transgenic mice Lab. Invest. 2020-07-01 [PMID: 32612285] (IF/IHC, Mouse)

Pearson G THE ROLE(S) OF LIPID SPECIES IN GLUCOSE-STIMULATED INSULIN SECRETION FROM PANCREATIC BETA-CELLS Thesis 2014-01-01 (WB, Mouse)

Lettieri Barbato D, Tatulli G, Aquilano K, Ciriolo MR. FoxO1 controls lysosomal acid lipase in adipocytes: implication of lipophagy during nutrient restriction and metformin treatment. Cell Death Dis. 2013-10-17 [PMID: 24136225] (WB, ICC/IF, Mouse)

## Procedures

### Western Blot protocol specific for LIPA antibody (NBP1-

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted 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 three 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.

\*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

### Immunohistochemistry-Paraffin protocol for Lipase A Antibody (NBP1-54155)

#### Immunohistochemistry-Paraffin Embedded Sections

##### Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

##### Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

**Immunocytochemistry/Immunofluorescence protocol for Lipase A Antibody (NBP1-54155)****Immunocytochemistry Protocol**

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.





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