Product Datasheet

CHREBP Antibody
NB400-135SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

Reviews: 4 Publications: 49

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Updated 6/15/2014 v.20.1

NB400-135SS

CHREBP Antibody

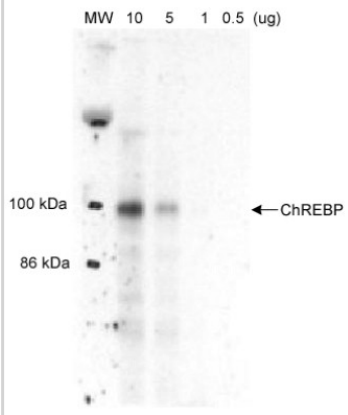
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.01% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-citrate/phosphate, pH 7-8
Target Molecular Weight	95 kDa

Product Description	
Host	Rabbit
Gene ID	51085
Gene Symbol	MLXIPL
Species	Human, Mouse, Rat
Species Reactivity	Human, rat and mouse (PMID 21835137).
Immunogen	A C-terminal synthetic peptide made to the human CHREBP protein sequence (between residues 800-852). [UniProt# Q9NP71, Isoform 1/Alpha]

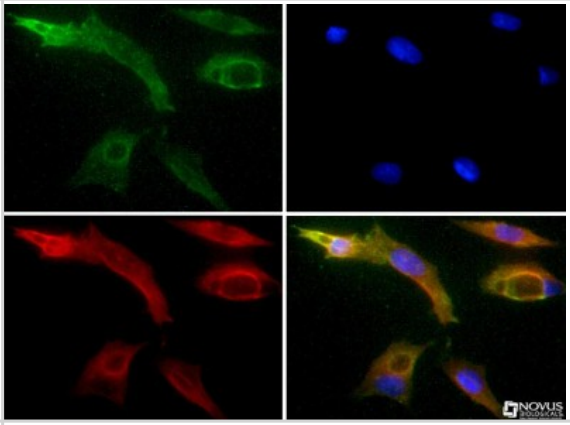
Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Chromatin Immunoprecipitation 1:10-1:500, Immunocytochemistry/Immunofluorescence 1:100-1:500, Immunohistochemistry 1:100, Immunohistochemistry-Paraffin 1:100, Immunoprecipitation 1:10-1:500, Western Blot 1:1000
Application Notes	This CHREBP antibody is useful in ChIP (PMID: 21282101), Immunohistochemistry paraffin embedded sections, Immunocytochemistry/Immunofluorescence and Western blot, where a band is seen at ~95 kDa.

Images

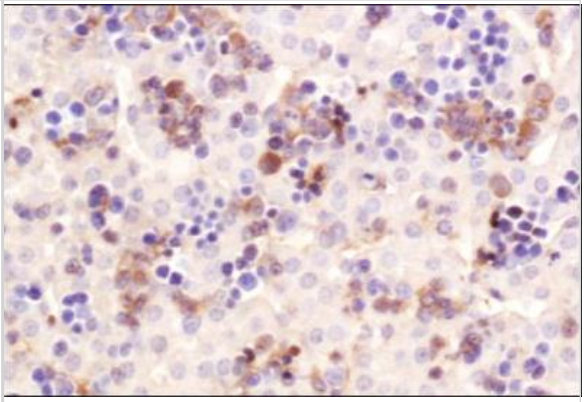
Western Blot: CHREBP Antibody [NB400-135] - Detection of ChREBP in liver nuclear extracts from well-fed rats. 7% SDS gel, 1:1,000 dilution of NB400-135. Photo courtesy of Dr. Uyeda, UT Southwestern University.



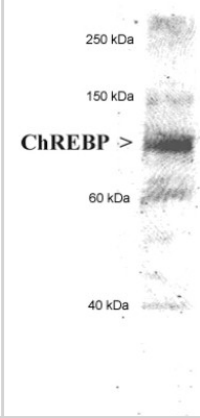
Immunocytochemistry/Immunofluorescence: CHREBP Antibody [NB400-135] - CHREBP antibody was tested in HepG2 cells with FITC (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



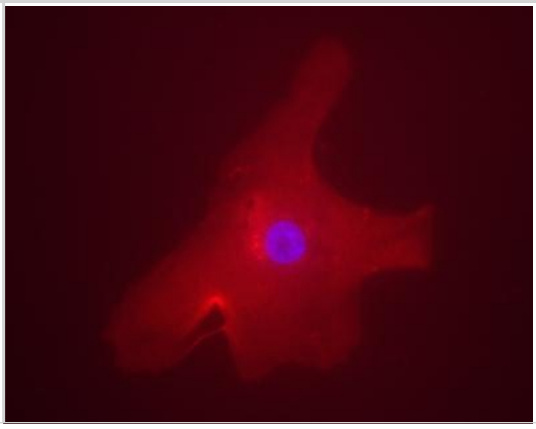
Immunohistochemistry: CHREBP Antibody [NB400-135] - Analysis of CHREBP in mouse liver using DAB with hematoxylin counterstain.



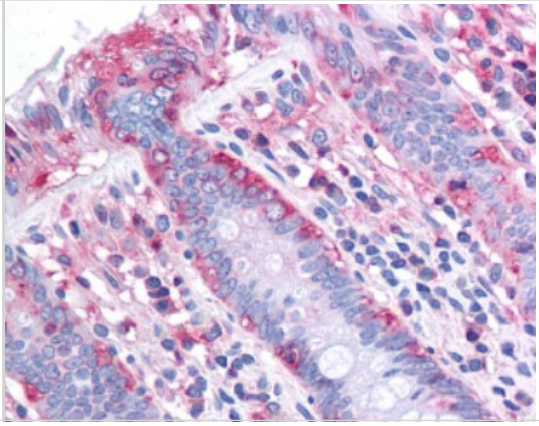
Western Blot: CHREBP Antibody [NB400-135] - Detection of ChREBP in 20 ug of human hepatocyte lysate using NB400-135. 5-10 second film exposure.



Immunocytochemistry/Immunofluorescence: CHREBP Antibody [NB400-135] - Immunofluorescent staining of a human hepatocyte using NB400-135. Overlay staining of ChREBP (rhodamine red) and DAPI nuclear staining (blue).



Immunohistochemistry: CHREBP Antibody [NB400-135] - Colon, Epithelium 40X.



Publications

Kaadige Mr, Yang J, Wilde Br, Ayer De. MondoA-Mlx transcriptional activity is limited by mTOR-MondoA interaction. Mol. Cell. Biol. 2014 Oct 20 [PMID: 25332233] (WB, Human)

Xing X, Li D, Chen D et al. Mangiferin treatment inhibits hepatic expression of acyl-coenzyme A:diacylglycerol acyltransferase-2 in fructose-fed spontaneously hypertensive rats: a link to amelioration of fatty liver. Toxicol. Appl. Pharmacol. 2014 Aug 11 [PMID: 25123789] (WB, Rat)

Chang ML, Chiu CJ, Shang F, Taylor A. High Glucose Activates ChREBP-Mediated HIF-1a and VEGF Expression in Human RPE Cells Under Normoxia. Adv. Exp. Med. Biol. 3/25/2014 [PMID: 24664750] (ChIP, WB, ICC/IF, Human)

Liu L, Yang M, Lin X et al. Modulation of hepatic sterol regulatory element-binding protein-1c-mediated gene expression contributes to Salacia oblonga root-elicited improvement of fructose-induced fatty liver in rats. J Ethnopharmacol. 2013 Oct 21 [PMID: 24157375] (WB, Rat)

Chambers KT, Chen Z, Lai L et al. PGC-1beta and ChREBP partner to cooperatively regulate hepatic lipogenesis in a glucose concentration-dependent manner. Mol Metab. 2013 May 9 [PMID: 24049734] (WB, ChIP, Mouse)

Jo SH, Kim MY, Park JM et al. Txnip contributes to impaired glucose tolerance by upregulating the expression of genes involved in hepatic gluconeogenesis in mice. Diabetologia. 2013 Sep 14 [PMID: 24037087] (ChIP, Mouse)

Wu L, Chen H, Zhu Y et al. Flightless I homolog negatively regulates ChREBP activity in cancer cells. Int J Biochem Cell Biol. 2013 Nov [PMID: 24055811] (IP, WB, Human)

Fukasawa M, Ge Q, Wynn RM et al. Coordinate regulation/localization of the carbohydrate responsive binding protein (ChREBP) by two nuclear export signal sites: discovery of a new leucine-rich nuclear export signal site. Biochem Biophys Res Commun. 2010 Jan [PMID: 20025850] (EMSA, Rat)

Liu C, Li Y, Zuo G et al. Oleanolic Acid Diminishes Liquid Fructose-Induced Fatty Liver in Rats: Role of Modulation of Hepatic Sterol Regulatory Element-Binding Protein-1c-Mediated Expression of Genes Responsible for De Novo Fatty Acid Synthesis. Evid Based Complement Alternat Med 2013 [PMID: 23737835] (WB, Rat)

Burke SJ, Collier JJ, Scott DK et al. cAMP prevents glucose-mediated modifications of histone H3 and recruitment of the RNA polymerase II holoenzyme to the L-PK gene promoter. J Mol Biol 2009 Sep 25 [PMID: 19631660] (ChIP, Rat)

Han S, Vaziri ND, Gollapudi P et al. Hepatic fatty acid and cholesterol metabolism in nephrotic syndrome Am J Transl Res 2013 [PMID: 23573368] (WB, Rat)

Liu Y, Major AS, Zienkiewicz J et al. Nuclear Transport Modulation Reduces Hypercholesterolemia, Atherosclerosis, and Fatty Liver J Am Heart Assoc 2013 Apr 5 [PMID: 23563994] (WB, Mouse)

More publications at http://www.novusbio.com/NB400-135

Procedures

Protocol specific for CHREBP Antibody (NB400-135)
Procedure for Western blot

1. Wash human hepatocytes grown in a culture dish with PBS twice and lyse the cells by Pierce M-Per reagent.

2. Measure protein concentration in lysates by Bio-rad protein assay.

3. Mix 20 ug of protein with Bio-rad loading buffer and 5% 2-mercaptoethanol. Boil for 5 minutes.

4. Place Bio-rad 4-15% Tris-Hcl gel. Load and run at 125V for 1 hour.

5. Transfer the proteins to nitrocellulose membrane. Run at 100V for 1 hour.

6. Block the membrane by 1% nonfat dry milk in Tris-buffered saline-Tween (TBS-T) for 1 hour.

7. Rinse the membrane twice by TBS-T.

8. Incubate with rabbit anti-ChREBP antibody (NB 400-135) 1:1,000-1:3,000 in TBS-T with 0.02% BSA overnight at 4 degrees C.

9. Wash the membrane twice by TBS-T.

10. Incubate with Goat anti-rabbit AP conjugated antibody 1:3000 in TBS-T for 1 hour at room temperature.

11. Wash the membrane twice by TBS-T.

12. Detect the signal by Bio-rad Immun-star kit and develop film.

Procedure for immunofluorescent staining

1. Wash human hepatocytes grown on cover-slips with PBS twice.

2. Fix cells with 10% buffered formalin for 25 minutes at room temperature.

3. Wash cells with PBS 3 times.

4. Incubate with 0.5% Triton X-100 in PBS for 5 minutes.

5. Wash cells with PBS 3 times.

6. Block with 1% BSA in PBS for 15 minutes at 37 degrees C.

7. Incubate with rabbit anti-ChREBP antibody (NB 400-135) 1:100-1:500 in PBS with 1% BSA for 30 minutes at 37 degrees C.

8. Wash cells with PBS 3 times.

9. Incubate with Goat anti-rabbit Alexa Fluor 568 antibody 1:2,000 in PBS with 1% BSA for 30 minutes at 37 degrees C.

10. Wash cells with PBS 3 times.

11. Mount by Vectashield mounting medium with DAPI.

12. Take pictures by using Rhodamine and DAPI filters.

I. Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

A. Place slides in peroxidase quenching solution: 15-30 minutes.

To Prepare 200 ml of Quenching Solution:

Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol. Use within 4 hours of preparation

B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees C.

B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.

C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.

D. Slowly add distilled water to further cool for 5 minutes.

E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).

B. Flood slide with Wash Solution.

Do not allow tissue sections to dry for the rest of the procedure.

C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.

D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.

E. Wash slides with Wash Solution: 3 changes for 5 minutes each.

F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.

G. Wash slides with Wash Solution: 3 changes for 5 minutes each.

H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.

I. Wash slides with Wash Solution: 3 changes for 5 minutes each.

J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired

K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.

L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.

M. Rinse slides in distilled water.

N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.

O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.

P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.

-Prior to deparaffinization, heat slides overnight in a 60 degrees C oven.

-All steps in which Xylene is used should be performed in a fume hood.

For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.