Datasheet for ABIN2780237

anti-NCOA4 antibody (N-Term)

1 Validation

1 Image

Go to Product page

Overview

Quantity:	100 µL
Target:	NCOA4
Binding Specificity:	N-Term
Reactivity:	Human, Mouse, Rat, Cow, Dog, Horse, Guinea Pig, Rabbit
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This NCOA4 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	The immunogen is a synthetic peptide corresponding to a region of Mouse
Sequence:	CLIHQLEYTQ NKDLANQVSV CLERLGSLAL KPEDSTVLLF EADTSALRQT
Predicted Reactivity:	Cow: 92%, Dog: 93%, Guinea Pig: 100%, Horse: 93%, Human: 100%, Mouse: 100%, Rabbit: 100%, Rat: 100%
Characteristics:	This is a rabbit polyclonal antibody against Ncoa4. It was validated on Western Blot.
Purification:	Affinity Purified

Target Details

Target:	NCOA4
Alternative Name:	Ncoa4 (NCOA4 Products)

Target Details

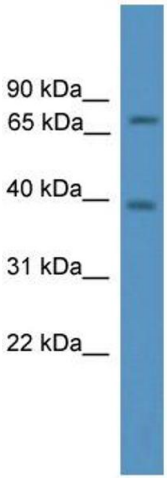
Background:	The function of Ncoa4 remains unknown. Alias Symbols: 1110034E15Rik, 4432406M01Rik, AI227008, ARA70, MGC103382, Rfg Protein Interaction Partner: Ndn, Protein Size: 625
Molecular Weight:	70 kDa
Gene ID:	27057
NCBI Accession:	NM_001033988, NP_001029160
Pathways:	Intracellular Steroid Hormone Receptor Signaling Pathway

Application Details

Application Notes:	Optimal working dilutions should be determined experimentally by the investigator.
Comment:	Antigen size: 625 AA
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	Lot specific
Buffer:	Liquid. Purified antibody supplied in 1x PBS buffer with 0.09 % (w/v) sodium azide and 2 % sucrose.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	For short term use, store at 2-8°C up to 1 week. For long term storage, store at -20°C in small aliquots to prevent freeze-thaw cycles.



Western Blotting

Image 1. WB Suggested Anti-Ncoa4 Antibody Titration: 0.2-1 ug/ml ELISA Titer: 1:312500 Positive Control: Mouse Heart

Validation report #100057 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by University of Michigan Medical School, Molecular & Integrative Physiology, Shah lab

Report Number: 100057

Date: Sep 12 2016

Target:	Ncoa4
Lot Number:	QC11327
Method validated:	Western Blotting (WB)
Positive Control:	HIF overexpression mice, WT mice
Negative Control:	NCOA4-/- mice
Notes:	Passed, based on the fact that the antibody picked up the positive control (unpublished data suggests that NCOA4 is highly expressed in an intestinal HIF overexpression model).
Primary Antibody:	ABIN2780237
Secondary Antibody:	Anti-rabbit HRP-linked secondary antibody (Cell Signaling, 7074S, lot 26)

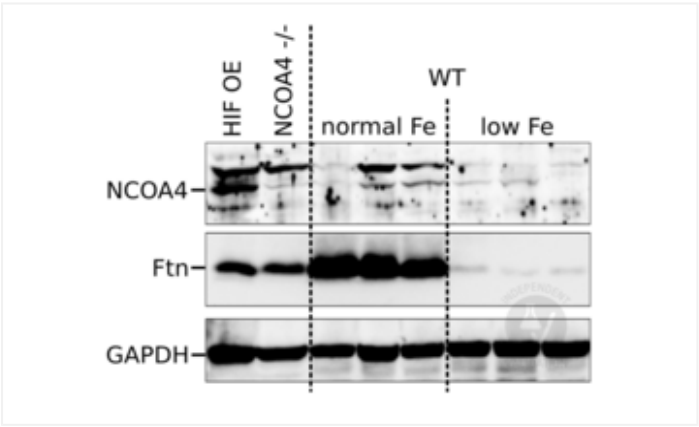
Protocol:	• Wild type (WT) or NCOA4 -/- mice were fed normal or iron-deficient diet for 7 days, followed by euthanization and scraping of duodenal epithelium (DE).
	• DE cells were lysed in RIPA buffer and whole cell lysate (WCL) prepared.
	• 50µg total protein from each sample were used for Western blot analysis.
	• Samples were denatured in 5x gel-loading buffer containing beta-marcaptoethanol and separated on a 10% SDS-PAGE gel (Laemmli 1970).
	• Transfer onto Nitrocellulose membrane (Biorad, 1620212, lot 10484060) was done on Trans-Blot SD apparatus (Bio-Rad) following the manufacturer's protocol.
	• Membranes were blocked with TBS-tween (TBST) solution containing 5% milk for 1h.
	• Incubation with primary antibody overnight at 4°C:
	  ○ NCOA4 antibody ABIN2780237 (lot QC11327) diluted 1:1000 in TBST containing 0.1% BSA.
	  ○ FTH1 antibody (Cell signaling, 3998S; lot 1) diluted in TBST containing 0.1% BSA.
	  ○ GAPDH antibody (Santa Cruz, SC-25778, lot D30115) diluted in TBST containing 0.1% BSA.
	• Washing with TBST for 15min at RT.
	• Incubation with anti-rabbit HRP-linked secondary antibody (Cell Signaling, 7074S, lot 26) diluted 1:2000 in TBST a shaker for 1h at RT.
	• Three rinses, followed by four 15min washes, twice TBST and twice with TBS.
	• Chemiluminescence detection was done using ECL Select Western blotting detection reagent (Amersham, RPN2235, lot 9723881).
	• Blot was developed on a Biorad imaging system.

Validation report #100057 for Western Blotting (WB)

Experimental Notes:

- The antibody did not consistently pick up the WT mice on normal and low iron diet.
- Also, there is still a faint band in the NCOA4 -/- lane. We are confident that it is a true KO mouse, as there is high ferritin expression even after 7 days of low iron diet (compared to ferritin expression in WT low iron mice as well as that of the HIF over expression mouse, which are known to have high intestinal ftn content).

Image for Validation report #100057



Validation image no. 1 for anti-Nuclear Receptor Coactivator 4 (NCOA4) (N-Term) antibody (ABIN2780237)

Mouse duodenum whole cell lysates from intestinal HIF overexpression (HIF OE) mice, NCOA4-/- mice, and wt mice fed a normal or low iron diet were separated by SDS-PAGE. NCOA4 was visualized using ABIN2780237. A ferritin (Ftn) Western blot was done to confirm the efficacy of iron diet regimens in mice. GAPDH served as loading control.