abcam

ab280082

Anti-FTO antibody [EPR24440-12] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR24440-12
Purification technique	Affinity purification Protein A
Concentration	1.022 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Mouse
Dilution info	-

Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Target data

See full target information FTO

Function

RNA demethylase that mediates oxidative demethylation of different RNA species, such as mRNAs, tRNAs and snRNAs, and acts as a regulator of fat mass, adipogenesis and energy homeostasis (PubMed:22002720, PubMed:25452335, PubMed:26457839, PubMed:26458103, PubMed:28002401, PubMed:30197295). Specifically demethylates N(6)-methyladenosine (m6A) RNA, the most prevalent internal modification of messenger RNA (mRNA) in higher eukaryotes (PubMed:22002720, PubMed:25452335, PubMed:26457839, PubMed:26458103, PubMed:30197295). M6A demethylation by FTO affects mRNA expression and stability (PubMed:30197295). Also able to demethylate m6A in U6 small nuclear RNA (snRNA) (PubMed:30197295). Mediates demethylation of N(6),2'-O-dimethyladenosine cap (m6A(m)), by demethylating the N(6)-methyladenosine at the second transcribed position of mRNAs and U6 snRNA (PubMed:28002401, PubMed:30197295). Demethylation of m6A(m) in the 5'-cap by FTO affects mRNA stability by promoting susceptibility to decapping (PubMed:28002401). Also acts as a tRNA demethylase by removing N(1)-methyladenine from various tRNAs (PubMed:30197295). Has no activity towards 1-methylguanine (PubMed:20376003). Has no detectable activity towards double-stranded DNA (PubMed:20376003). Also able to repair alkylated DNA and RNA by oxidative demethylation: demethylates single-stranded RNA containing 3-methyluracil, single-stranded DNA containing 3-methylthymine and has low demethylase activity towards single-stranded DNA containing 1-methyladenine or 3-methylcytosine (PubMed:18775698, PubMed:20376003). Ability to repair alkylated DNA and RNA is however unsure in vivo (PubMed:18775698, PubMed:20376003). Involved in the regulation of fat mass, adipogenesis and body weight, thereby contributing to the regulation of body size and body fat accumulation (PubMed:18775698, PubMed:20376003). Involved in the regulation of thermogenesis and the control of adipocyte differentiation into brown or white fat cells (PubMed:26287746). Regulates activity

of the dopaminergic midbrain circuitry via its ability to demethylate m6A in mRNAs (By similarity). Plays an oncogenic role in a number of acute myeloid leukemias by enhancing leukemic oncogene-mediated cell transformation: acts by mediating m6A demethylation of target transcripts such as MYC, CEBPA, ASB2 and RARA, leading to promote their expression (PubMed:28017614, PubMed:29249359).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab280082 is the carrier-free version of ab280081.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The FTO protein also referred to as Alpha-Ketoglutarate-Dependent Dioxygenase or Fat Mass and Obesity-Associated protein functions as an enzyme involved in demethylating nucleic acids specifically N6-methyladenosine (m6A) in RNA. This process impacts RNA stability and translation. FTO has a molecular weight of approximately 58 kDa. Higher expression levels of FTO are found in the brain including the hypothalamus which is an area known for regulating energy balance and appetite.
Biological function summary	FTO influences energy homeostasis and adipogenesis. It acts as an important player in metabolic regulation and gene expression by modulating the m6A RNA modification. While FTO operates largely on its own its demethylation activity may interact with other RNA binding proteins that recognize m6A-modified RNAs integrating it into larger regulatory frameworks for RNA metabolism and cellular responses.
Pathways	FTO is an important component in the regulation of energy balance and metabolism. The protein is closely associated with the mTOR signaling pathway which is essential for controlling cell growth proliferation and survival in response to nutrient availability. FTO also interacts with the leptin signaling pathway relating to its role in appetite regulation and energy expenditure.
Associated diseases and disorders	FTO has significant connections to obesity and Type 2 diabetes. Genetic variants within the FTO gene correlate with risk for these conditions linking its enzymatic activity to alterations in energy balance and insulin sensitivity. FTO's role in Type 2 diabetes also highlights its interaction with insulin signaling proteins demonstrating its broader influence on glucose metabolism.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

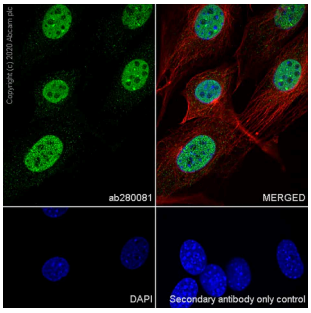
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

6 product images



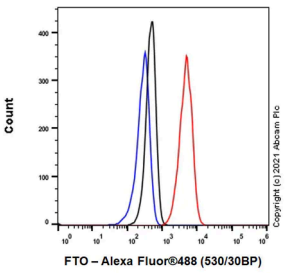
Immunocytochemistry/ Immunofluorescence - Anti-FTO antibody [EPR24440-12] - BSA and Azide free (ab280082)

FTO Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-FTO antibody

This data was developed using ab280081, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling FTO with ab280081 at 1/50 (11.98 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear and weak cytoplasmic staining in NIH/3T3 cell line is observed.

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

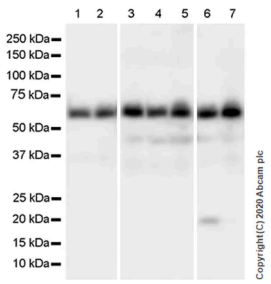
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-FTO antibody [EPR24440-12] - BSA and Azide free (ab280082)

FTO Flow Cytometry (Intracellular) staining using rabbit Anti-FTO antibody

This data was developed using ab280081, the same antibody clone in a different buffer formulation.Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling FTO with ab280081 at 1/500 dilution (0.1ug)/ (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-FTO antibody [EPR24440-12] - BSA and Azide free (ab280082)

FTO Western blot staining using rabbit Anti-FTO antibody

This data was developed using ab280081, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: Lanes 1-2:15 seconds; Lanes 3-5: 6 seconds; Lanes:6-7:15 seconds.

All lanes:
Western blot - Anti-FTO antibody [EPR24440-12] (ab280081) at 1/1000 dilution

Lane 1:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 2:
C2C12 (mouse myoblasts myoblast) whole cell lysate at 20 µg

Lane 3:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 4:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
HAP1 (human chronic myelogenous leukemia near-haploid cell line) whole cell lysate at 20 µg

Lane 6:
PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

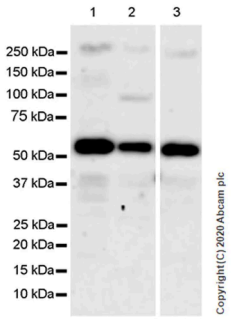
Lane 7:
L6 (rat skeletal muscle myoblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 58 kDa

Observed band size: 60 kDa



Western blot - Anti-FTO antibody [EPR24440-12] - BSA and Azide free (ab280082)

FTO Western blot staining using rabbit Anti-FTO antibody

This data was developed using ab280081, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 3 minutes.

All lanes:
Western blot - Anti-FTO antibody [EPR24440-12] (ab280081) at 1/1000 dilution

Lane 1:
Mouse brain tissue lysate at 20 µg

Lane 2:
Mouse heart tissue lysate at 20 µg

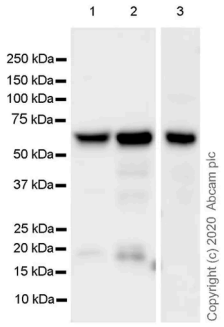
Lane 3:
Rat brain tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 58 kDa

Observed band size: 60 kDa



Western blot - Anti-FTO antibody [EPR24440-12] - BSA and Azide free (ab280082)

FTO Western blot staining using rabbit Anti-FTO antibody

This data was developed using ab280081, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: Lanes 1-2: 15 seconds;Lane 3: 48 seconds.

All lanes:
Western blot - Anti-FTO antibody [EPR24440-12] (ab280081) at 1/1000 dilution

Lane 1:
Human lung tissue lysate 20 at 20 µg

Lane 2:
Human kidney tissue lysate at 20 µg

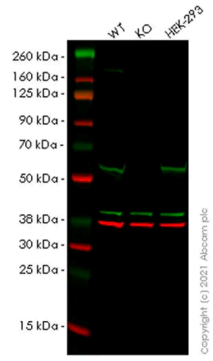
Lane 3:
Human skeletal muscle tissue lysate at 20 µg

Secondary

All lanes:
Western blot - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

Predicted band size: 58 kDa

Observed band size: 60 kDa



Western blot - Anti-FTO antibody [EPR24440-12] - BSA and Azide free (ab280082)

FTO Western blot staining using rabbit Anti-FTO antibody

This data was developed using ab280081, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: Lanes 1-3: Merged signal (red and green). Green - ab280081 observed at 60 kDa. Red - loading control ab8245 observed at 36 kDa.

Blocking and diluting buffer and concentration: 3% NFDM/TBST

Lanes 1-3: Merged signal (red and green). Green - ab280081 observed at 60 kDa. Red - loading control ab8245 observed at 36 kDa.

ab280081 Anti-FTO antibody [EPR24440-12] was shown to specifically react with FTO in wild-type HAP1 cells. Loss of signal was observed when the knockout cell line was used. Wild-type and FTO knockout samples were subjected to SDS-PAGE. ab280081 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4☒ overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-FTO antibody [EPR24440-12] (ab280081) at 1/1000 dilution

Lane 1:

Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 20 µg

Lane 2:
FTO knockout HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 20 µg

Lane 3:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/10000 dilution

Predicted band size: 58 kDa

Observed band size: 60 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.