

Product datasheet

Anti-FTO antibody [EPR24440-12] ab280081

KO VALIDATED Recombinant RabMAb[®]

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Overview

Product name	Anti-FTO antibody [EPR24440-12]
Description	Rabbit monoclonal [EPR24440-12] to FTO
Host species	Rabbit
Tested applications	Suitable for: WB, Flow Cyt (Intra), ICC/IF Unsuitable for: IHC-P or IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1, HEK-293, NIH/3T3, C2C12, HeLa, PC-12, L6, Human lung, Human kidney, Human skeletal muscle, Mouse brain and Mouse heart, Rat brain lysates. ICC/IF: NIH/3T3 cells. Flow Cyt(intra): NIH/3T3 cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24440-12

Isotype

IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab280081 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Predicted molecular weight: 58 kDa.
Flow Cyt (Intra)		1/500.
ICC/IF		1/50.

Application notes Is unsuitable for IHC-P or IP.

Target

Function Dioxygenase that repairs alkylated DNA and RNA by oxidative demethylation. Has highest activity towards single-stranded RNA containing 3-methyluracil, followed by single-stranded DNA containing 3-methylthymine. Has low demethylase activity towards single-stranded DNA containing 1-methyladenine or 3-methylcytosine. Has no activity towards 1-methylguanine. Has no detectable activity towards double-stranded DNA. Requires molecular oxygen, alpha-ketoglutarate and iron. Contributes to the regulation of the global metabolic rate, energy expenditure and energy homeostasis. Contributes to the regulation of body size and body fat accumulation.

Tissue specificity Ubiquitously expressed, with relatively high expression in adrenal glands and brain; especially in hypothalamus and pituitary.

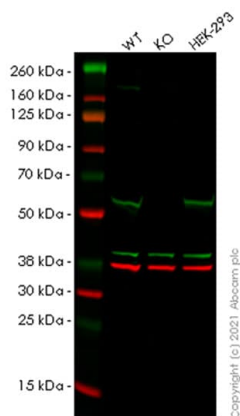
Involvement in disease Defects in FTO are the cause of growth retardation developmental delay coarse facies and early death (GRDDCFED) [MIM:612938]. The disease consists of a severe children multiple congenital anomaly syndrome with death by the age of 3 years. All affected individuals had postnatal growth retardation, microcephaly, severe psychomotor delay, functional brain deficits, and characteristic facial dysmorphism. In some patients, structural brain malformations, cardiac defects, genital anomalies, and cleft palate were also observed.

Sequence similarities Belongs to the fto family.

Domain The 3D-structure of the Fe2OG dioxygenase domain is similar to that of the Fe2OG dioxygenase domain found in the bacterial DNA repair dioxygenase alkB and its mammalian orthologs, but sequence similarity is very low. As a consequence, the domain is not detected by protein signature databases.

Cellular localization Nucleus.

Images



Western blot - Anti-FTO antibody [EPR24440-12] (ab280081)

All lanes : 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

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

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

Lysates/proteins at 20 µg per lane.

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

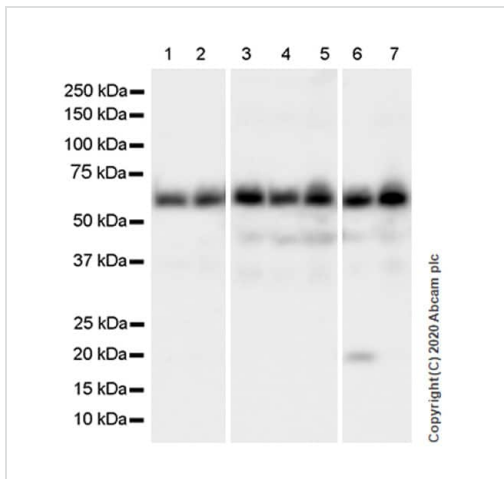
Predicted band size: 58 kDa

Observed band size: 60 kDa

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°C 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.



Western blot - Anti-FTO antibody [EPR24440-12] (ab280081)

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

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

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

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

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

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

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

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

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

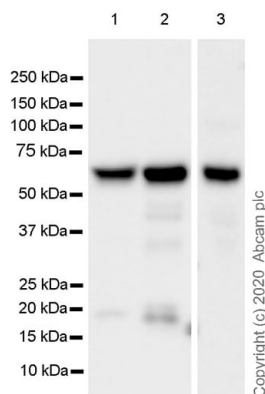
Predicted band size: 58 kDa

Observed band size: 60 kDa

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.



Western blot - Anti-FTO antibody [EPR24440-12]
(ab280081)

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

Lane 1 : Human lung tissue lysate 20

Lane 2 : Human kidney tissue lysate

Lane 3 : Human skeletal muscle tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

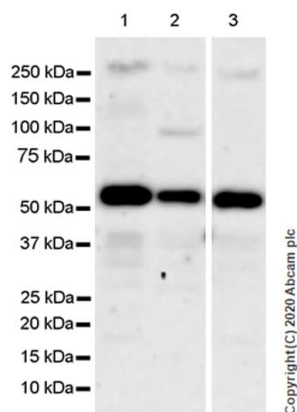
All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/1000 dilution (VeriBlot for IP secondary antibody(HRP))

Predicted band size: 58 kDa

Observed band size: 60 kDa

Blocking and diluting buffer and concentration: 5% NFDm/TBST

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



Western blot - Anti-FTO antibody [EPR24440-12] (ab280081)

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

Lane 1 : Mouse brain tissue lysate

Lane 2 : Mouse heart tissue lysate

Lane 3 : Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

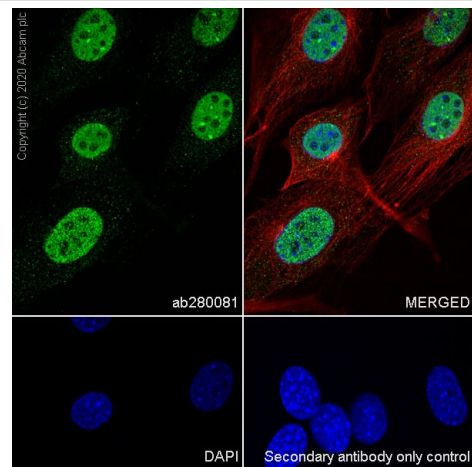
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 58 kDa

Observed band size: 60 kDa

Blocking and diluting buffer and concentration: 5% NFD/MTBST

Exposure time: 3 minutes.

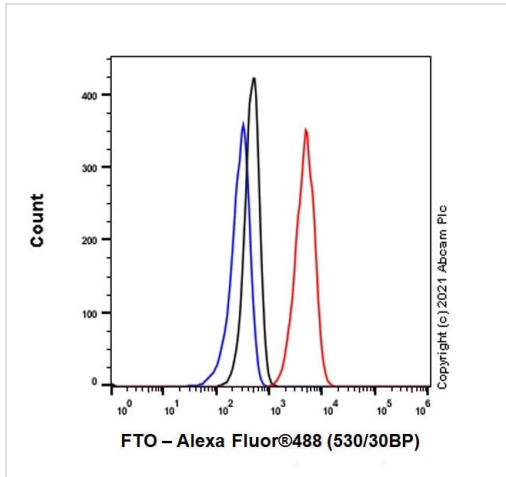


Immunocytochemistry/ Immunofluorescence - Anti-FTO antibody [EPR24440-12] (ab280081)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling FTO with ab280081 at 1/50 (11.98 µg/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 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.

Flow Cytometry (Intracellular) - Anti-FTO antibody [EPR24440-12] (ab280081)

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-FTO antibody [EPR24440-12] (ab280081)

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