abcam

Product datasheet

Anti-FTO antibody [EPR6894] ab126605

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-FTO antibody [EPR6894]
Description	Rabbit monoclonal [EPR6894] to FTO
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human FTO (N terminal). The exact sequence is proprietary.
Positive control	WB: Wild-type MCF7, HEK-293, MOLT-4, BxPC-3, Caco-2, SH-SY5Y and 293T cell lysates. IHC-P: Human hepatocellular carcinoma and human adrenal gland tissues. ICC/IF: BxPC-3 cells.
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 59% PBS, 40% Glycerol
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR6894
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab126605 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		1/10000 - 1/20000. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa). **For unpurified, use 1/1000 - 1/10000.**
IHC-P	★★★★☆ **(1)**	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **For unpurified, use 1/50 - 1/100.** See **IHC antigen retrieval protocols**.
ICC/IF	★★★★★ **(1)**	1/250. **For unpurified, use 1/50 - 1/100.**

Application notes Is unsuitable for Flow Cyt.

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.

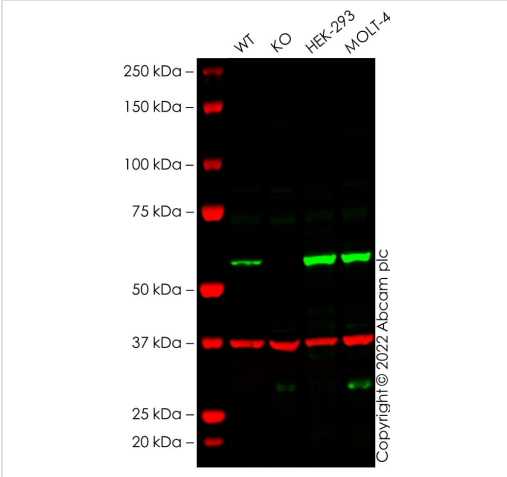
Involvement in disease Defects in FTO are the cause of growth retardation developmental delay coarse facies and early death (GRDDCFED) [MIM:612938]. The disease consists of a severe children multiple congenital anomaly syndrome with death by the age of 3 years. All affected individuals had postnatal 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 [EPR6894] (ab126605)

All lanes : Anti-FTO antibody [EPR6894] (ab126605) at 1/1000 dilution

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : FTO knockout MCF7 cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : MOLT-4 cell lysate

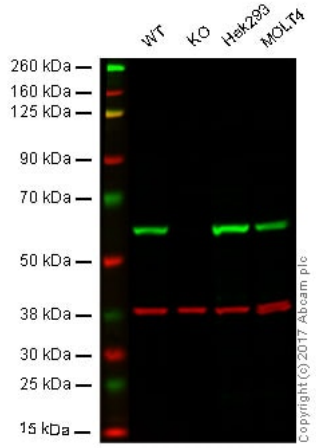
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 58 kDa

False colour image of Western blot: Anti-FTO antibody [EPR6894] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab126605 was shown to bind specifically to FTO. A band was observed at 58 kDa in wild-type MCF7 cell lysates with no signal observed at this size in FTO knockout cell line. To generate this image, wild-type and FTO knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

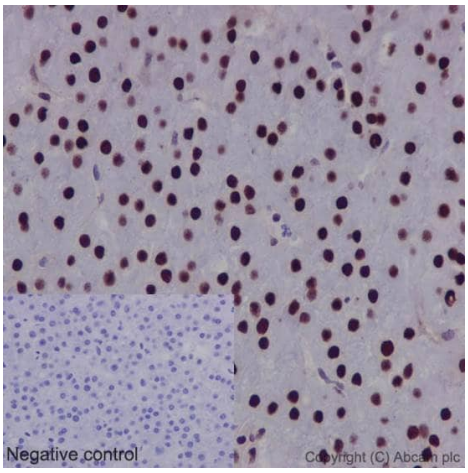


Western blot - Anti-FTO antibody [EPR6894] (ab126605)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: FTO knockout HAP1 whole cell lysate (20 µg)
Lane 3: HEK293 whole cell lysate (20 µg)
Lane 4: MOLT4 whole cell lysate (20 µg)

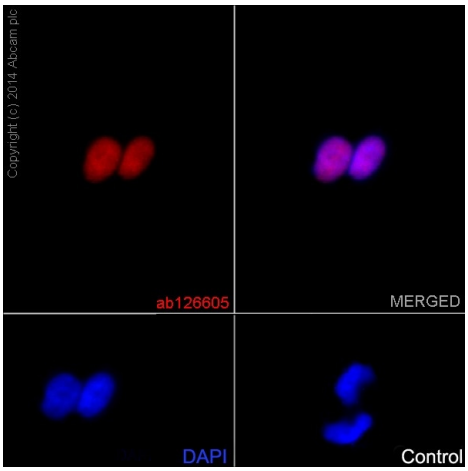
Lanes 1 - 4: Merged signal (red and green). Green - ab126605 observed at 58 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab126605 was shown to specifically react with FTO in wild-type HAP1 cells. No band was observed when FTO knockout samples were examined. Wild-type and FTO knockout samples were subjected to SDS-PAGE. ab126605 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/1000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



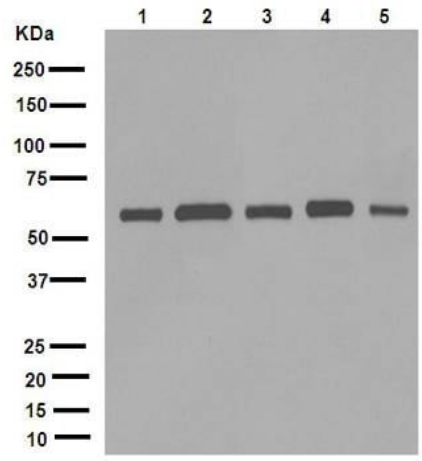
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FTO antibody [EPR6894] (ab126605)

Immunohistochemical staining of paraffin embedded human hepatocellular carcinoma with purified ab126605 at a working dilution of 1 in 500. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-FTO antibody [EPR6894] (ab126605)

Immunofluorescence staining of BxPC-3 cells with purified ab126605 at a working dilution of 1 in 250, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 555 goat anti rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, purified ab126605 was used at a dilution of 1/200 followed by an Alexa Fluor® 488 goat anti-mouse antibody at a dilution of 1/500.



Western blot - Anti-FTO antibody [EPR6894] (ab126605)

All lanes : Anti-FTO antibody [EPR6894] (ab126605) at 1/10000 dilution (purified)

Lane 1 : Molt-4 cell lysate
Lane 2 : HEK293 cell lysate
Lane 3 : BxPC-3 cell lysate
Lane 4 : Caco-2 cell lysate
Lane 5 : SH-SY5Y cell lysate

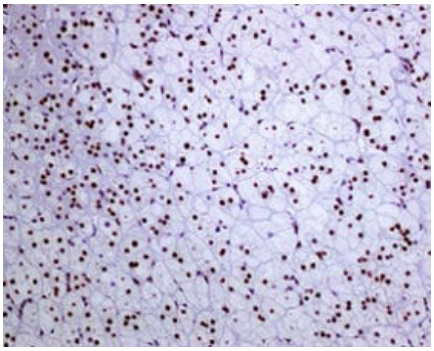
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 58 kDa
Observed band size: 58 kDa

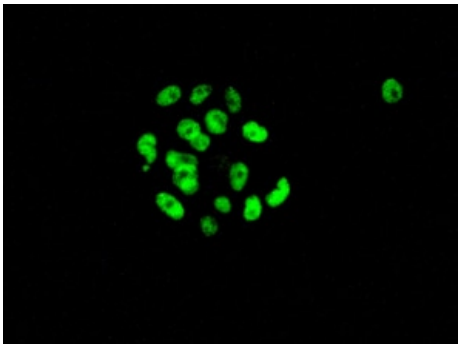
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



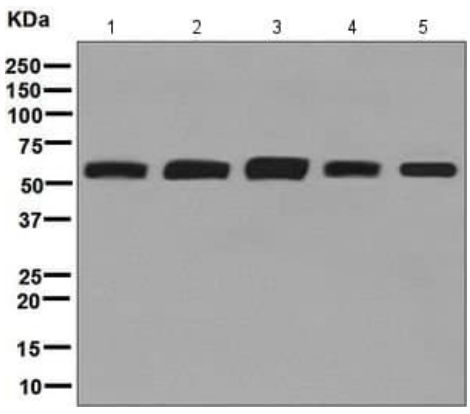
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FTO antibody [EPR6894] (ab126605)

Immunohistochemical staining of FTO in paraffin embedded human adrenal gland tissue using unpurified ab126605 at a 1/50 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-FTO antibody [EPR6894] (ab126605)

Unpurified ab126605 at a 1/50 dilution staining FTO in BxPC3 cells by immunofluorescence.



Western blot - Anti-FTO antibody [EPR6894] (ab126605)

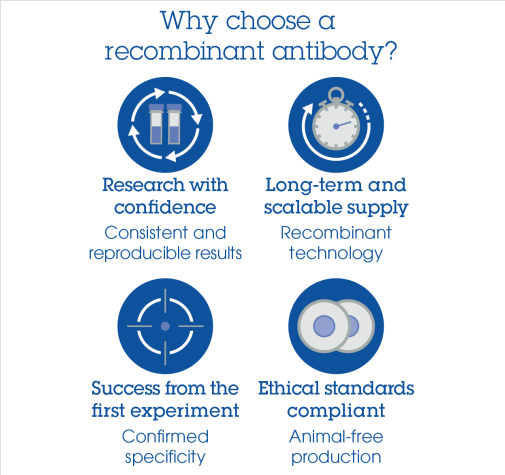
All lanes : Anti-FTO antibody [EPR6894] (ab126605) at 1/1000 dilution (unpurified)

Lane 1 : MOLT4 cell lysate
Lane 2 : 293T cell lysate
Lane 3 : SH SY5Y cell lysate
Lane 4 : BxPC3 cell lysate
Lane 5 : Caco 2 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

Predicted band size: 58 kDa
Observed band size: 58 kDa



Anti-FTO antibody [EPR6894] (ab126605)

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