abcam

Product datasheet

Anti-FTO antibody [EPR6895] ab124892

KO VALIDATED Recombinant RabMAb

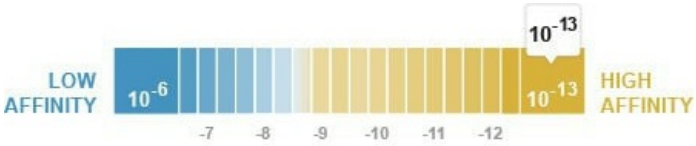
2 Abreviews 42 References 10 Images

Overview

Product name	Anti-FTO antibody [EPR6895]
Description	Rabbit monoclonal [EPR6895] to FTO
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse, Rat
Immunogen	Synthetic peptide within Human FTO aa 450-550. The exact sequence is proprietary.
Positive control	IHC-P: Human Breast, lung carcinoma and kidney tissues; WB: 293T, SH-SY5Y, Caco2, HEK-293 and BxPC3 lysates.
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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Dissociation constant (K_D)	K_D = 9.30 x 10^{-13} M



Learn more about K_D

Storage buffer	pH: 7.2

Preservative: 0.01% Sodium azide
Constituents: PBS, 0.05% BSA, 40% Glycerol

Purity Protein A purified

Clonality Monoclonal

Clone number EPR6895

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab124892 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)	1/1000 - 1/10000. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt,ICC/IF 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.

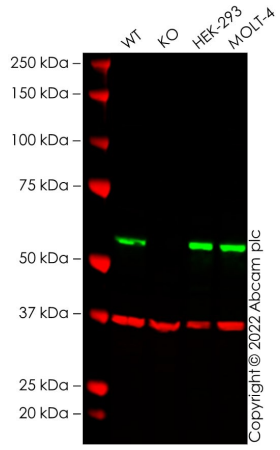
Involvement in disease Defects in FTO are the cause of growth retardation developmental delay coarse facies and early death (GRDDCFED) [MIM:612938]. The disease consists of a severe children multiple congenital anomaly syndrome with death by the age of 3 years. All affected individuals had postnatal 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 [EPR6895] (ab124892)

All lanes : Anti-FTO antibody [EPR6895] (ab124892) at 1/1000 dilution

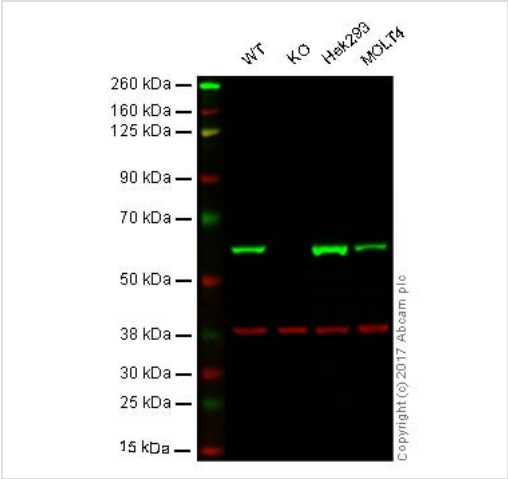
Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : FTO knockout MCF7 cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

False colour image of Western blot: Anti-FTO antibody [EPR6895] 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, ab124892 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 [EPR6895] (ab124892)

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 - ab124892 observed at 58 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab124892 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. ab124892 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.

Western blot - Anti-FTO antibody [EPR6895] (ab124892)

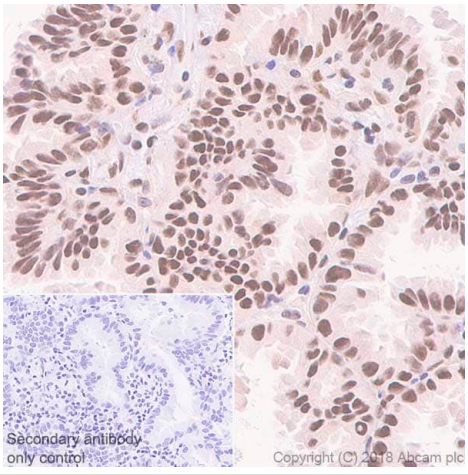
All lanes : Anti-FTO antibody [EPR6895] (ab124892) at 1/1000 dilution (Purified)

Lane 1 : Caco-2 (Human colorectal adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

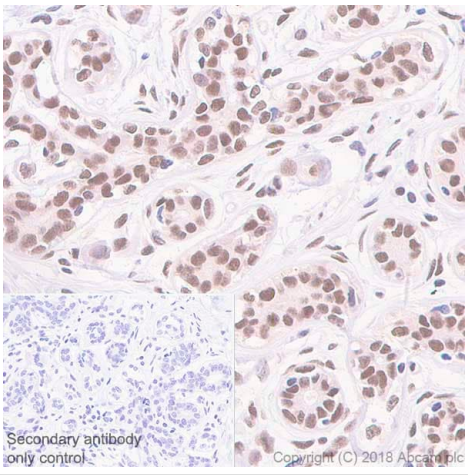
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



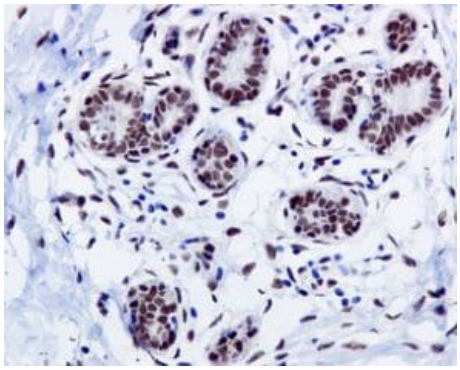
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FTO antibody [EPR6895] (ab124892)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung carcinoma tissue sections labeling FTO with purified ab124892 at 1:100 dilution (1.66 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use)was used as the secondary antibody. Negative control:PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



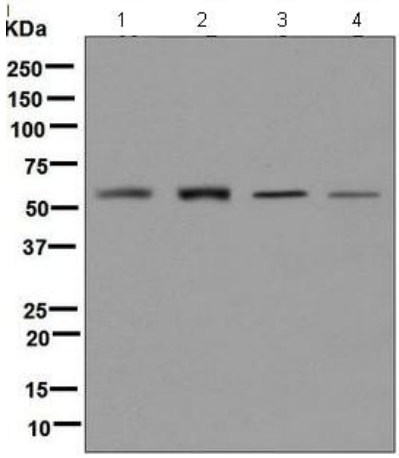
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FTO antibody [EPR6895] (ab124892)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast tissue sections labeling FTO with purified ab124892 at 1:100 dilution (1.66 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use)was used as the secondary antibody. Negative control:PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FTO antibody [EPR6895] (ab124892)

ab124892, at 1/100 dilution, staining FTO in Paraffin-embedded Human Breast tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Western blot - Anti-FTO antibody [EPR6895] (ab124892)

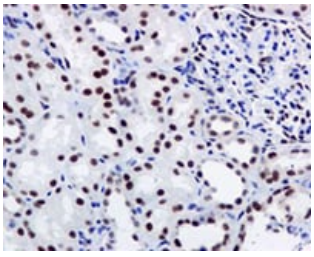
All lanes : Anti-FTO antibody [EPR6895] (ab124892) at 1/1000 dilution

Lane 1 : 293T lysate
Lane 2 : SH-SY5Y lysate
Lane 3 : Caco2 lysate
Lane 4 : BxPC3 lysate

Lysates/proteins at 10 µg per lane.

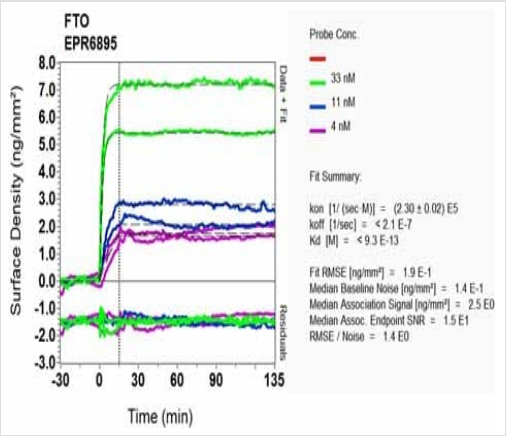
Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

Predicted band size: 58 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FTO antibody [EPR6895] (ab124892)

ab124892, at 1/100 dilution, staining FTO in Paraffin-embedded Human kidney tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



OI-RD Scanning - Anti-FTO antibody [EPR6895] (ab124892)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-FTO antibody [EPR6895] (ab124892)

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