


## Product datasheet

### Anti-FTO antibody [5-2H10] ab92821

KO VALIDATED

★★★★☆ 3 Abreviews 42 References 4 Images

#### Overview

Product name	Anti-FTO antibody [5-2H10]
Description	Mouse monoclonal [5-2H10] to FTO
Host species	Mouse
Tested applications	<b>Suitable for:</b> WB, ICC/IF, Flow Cyt
Species reactivity	<b>Reacts with:</b> Rat, Human <b>Predicted to work with:</b> Sheep, Horse, Cow, Dog, Non human primates 
Immunogen	Synthetic peptide corresponding to Human FTO (N terminal) conjugated to keyhole limpet haemocyanin. Database link: <a href="#">Q9C0B1</a>
Positive control	WB: Rat testes nuclear lysate. ICC/IF: Neuronal progenitor cells. Flow Cyt: SH-SY5Y cells.
General notes	<p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&amp;As</p>

#### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	Constituents: 0.238% HEPES, 0.01% BSA, 50% Glycerol, 0.87% Sodium chloride
Purity	Protein G purified
Purification notes	Protein G purified culture supernatant.
Clonality	Monoclonal
Clone number	5-2H10
Isotype	IgG2a

## Applications

### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab92821 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. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa).
ICC/IF	★★★★★ (1)	1/100.
Flow Cyt		Use 1µg for 10 <sup>6</sup> cells. <b>ab170191</b> - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

## 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.

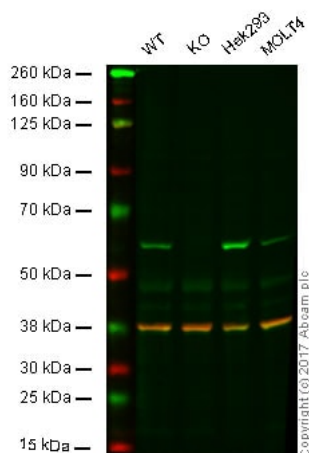
### Domain

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 [5-2H10]  
(ab92821)

**Lane 1:** Wild-type HAP1 whole cell lysate (20  $\mu$ g)

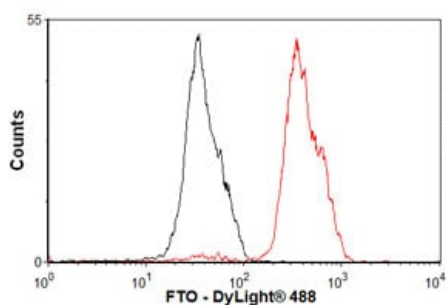
**Lane 2:** FTO knockout HAP1 whole cell lysate (20  $\mu$ g)

**Lane 3:** HEK293 whole cell lysate (20  $\mu$ g)

**Lane 4:** MOLT4 whole cell lysate (20  $\mu$ g)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab92821 observed at 58 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

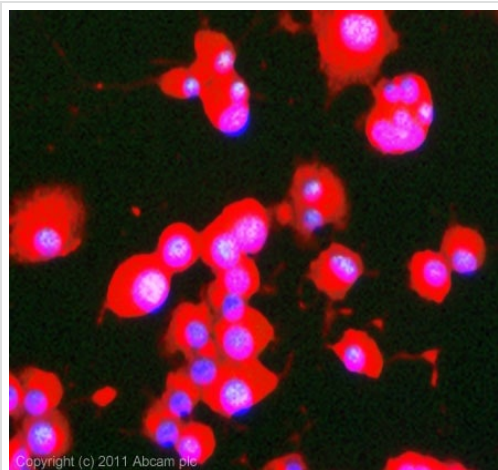
ab92821 was shown to specifically recognize FTO in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when FTO knockout samples were examined. Wild-type and FTO knockout samples were subjected to SDS-PAGE. ab92821 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at a 1/1000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry - Anti-FTO antibody [5-2H10]  
(ab92821)

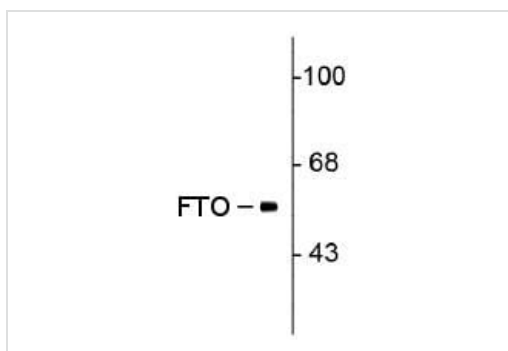
Overlay histogram showing SH-SY5Y cells stained with ab92821 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92821, 1 $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIG2A] (**ab91361**, 1 $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions.

Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-FTO antibody [5-2H10] (ab92821)

ICC/IF image of ab92821 stained PC12 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab92821, 5µg/ml) overnight at +4°C. The secondary antibody (green) was [ab96879](#), DyLight® 488 goat anti-mouse IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-FTO antibody [5-2H10] (ab92821)

Anti-FTO antibody [5-2H10] (ab92821) at 1/1000 dilution + Rat testes lysate

**Predicted band size:** 58 kDa

**Observed band size:** 58 kDa

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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