Product datasheet

Anti-FTO antibody [5-2H10] ab92821

Overview

Product name          Anti-FTO antibody [5-2H10]
Description           Mouse monoclonal [5-2H10] to FTO
Host species          Mouse
Tested applications   Suitable for: WB, ICC/IF, Flow Cyt, IHC-P
Species reactivity    Reacts with: Mouse, Rat, Human
Immunogen             Synthetic peptide from Human FTO.

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

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td></td>
<td>1/1000. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa).</td>
</tr>
</tbody>
</table>
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
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 - 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.

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, 1µg/1x10^6 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 [ICIGG2A] (ab91361, 1µg/1x10^6 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 AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors