abcam

Product datasheet

Anti-CAD antibody [EP710Y] ab40800



9 References 8 Images

Overview

Product name Anti-CAD antibody [EP710Y]

Description Rabbit monoclonal [EP710Y] to CAD

Host species Rabbit

Specificity ab40800 is specific to the N-terminus of CAD.

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

WB: HeLa and HEK293 cell lysate. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa Positive control

whole cell lysate (ab150035).

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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Protein A purified **Purity**

Clonality Monoclonal
Clone number EP710Y
Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab40800 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
Flow Cyt (Intra)		1/120.
ICC/IF		1/100 - 1/300.
WB		1/1000. Predicted molecular weight: 243 kDa.
IP		1/30 - 1/50.

Target

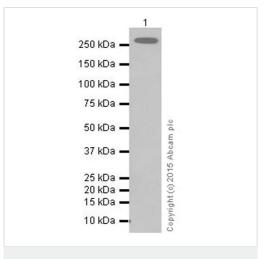
Relevance

Carbamoyl phosphate synthetase-aspartate carbamoyltransferase-dihydroorotase (CAD) is a multifunctional protein that initiates and regulates mammalian de novo pyrimidine biosynthesis. This trifunctional protein which is associated with the enzymatic activities of the first 3 enzymes in the 6-step pathway of pyrimidine biosynthesis is the rate-limiting step in the de novo pyrimidine synthetic pathway. Although most of the CAD protein in the cell is cytosolic, phosphorylation at threonine 456 localizes the protein to the nucleus. While MAPK and EGF phosphorylate CAD at threonine 456, MAPK and c-myc have been found to induce over-expression of CAD.

Cellular localization

Cytoplasmic and Nuclear

Images



Western blot - Anti-CAD antibody [EP710Y] (ab40800)

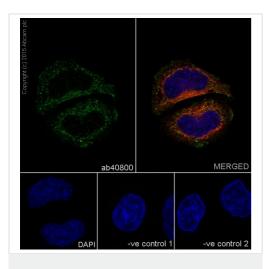
Anti-CAD antibody [EP710Y] (ab40800) at 1/2000 dilution (purified) + HeLa cell lysate at 10 µg

Secondary

HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

Predicted band size: 243 kDa **Observed band size:** 250 kDa

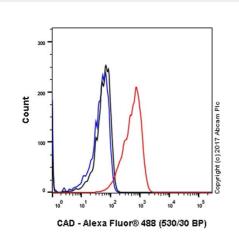
Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



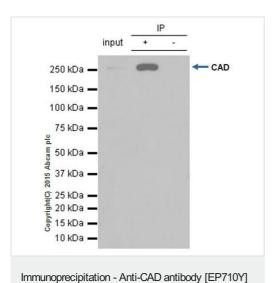
Immunocytochemistry/ Immunofluorescence - Anti-CAD antibody [EP710Y] (ab40800)

Immunofluorescence staining of HeLa cells with purified ab40800 at a working dilution of 1/300, counter-stained with DAPI. The secondary antibody was Alexa Fluor[®] 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse antitubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor[®] 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab40800 was used at a dilution of 1/500 followed by an Alexa Fluor[®] 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse antitubulin) was used at a dilution of 1/500 followed by an Alexa Fluor[®] 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling CAD (red) with ab40800 at a 1/120 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit lgG (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal lgG (ab172730). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.



Flow Cytometry (Intracellular) - Anti-CAD antibody [EP710Y] (ab40800)



ab40800 (purified) at 1/30 immunoprecipitating CAD in 10 μ g HeLa (Lanes 1 and 2, observed at 250 kDa). Lane 3 - PBS. For western blotting, HRP Veriblot for IP (**ab131366**) was used for detection (1/10 000). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST

(ab40800) 250 kDa —

Anti-CAD antibody [EP710Y] (ab40800) at 1/1000 dilution (purified) + HeLa cell lysate at 1/20000 dilution

Secondary

HRP goat anti-rabbit lgG (H+L) at 1/20000 dilution

Predicted band size: 243 kDa **Observed band size:** 250 kDa

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

37 kDa —

25 kDa —

20 kDa —

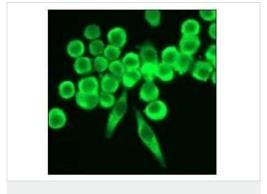
15 kDa —

15 kDa —

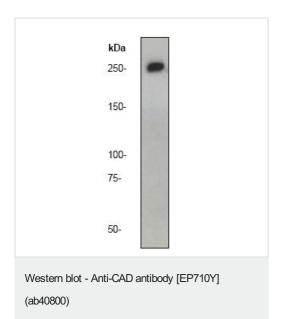
10 kDa —

Western blot - Anti-CAD antibody [EP710Y] (ab40800)

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

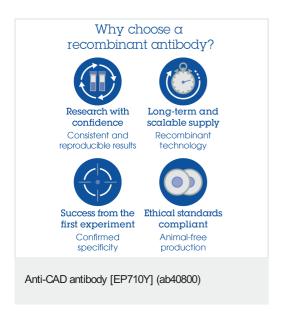


Immunocytochemistry/ Immunofluorescence - Anti-CAD antibody [EP710Y] (ab40800) Unpurified ab40800 (1/100), staining human CAD (N-term) in HeLa cells by Immunofluorescence.



Anti-CAD antibody [EP710Y] (ab40800) at 1/1000 dilution (unpurified) + HEK-293 whole cell lysate (ab7902) at 10 µg

Predicted band size: 243 kDa **Observed band size:** 250 kDa



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