

## **Product datasheet**

# Anti-CAD antibody [EP710Y] - BSA and Azide free ab231694

Recombinant RabMAb

### 8 Images

Overview	
Product name	Anti-CAD antibody [EP710Y] - BSA and Azide free
Description	Rabbit monoclonal [EP710Y] to CAD - BSA and Azide free
Host species	Rabbit
Specificity	This antibody is specific to the N-terminus of CAD.
Tested applications	Suitable for: ICC/IF, WB, IP, Flow Cyt (Intra)
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab231694 is the carrier-free version of <b>ab40800</b> .
	Our <b><u>carrier-free</u></b> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.
	This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.
	Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.
	This product is compatible with the Maxpar <sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar <sup>®</sup> is a trademark of Fluidigm Canada Inc.
	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>
	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

Pro	perties
	201000

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP710Y
lsotype	lgG

#### Applications

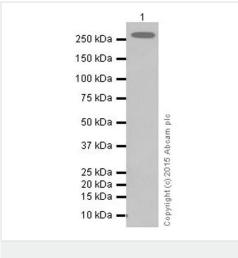
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab231694 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

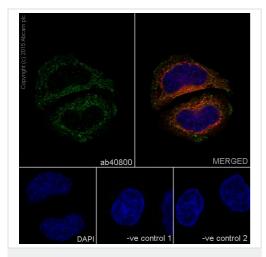
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 243 kDa.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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] - BSA and Azide free (ab231694)



Immunocytochemistry/ Immunofluorescence - Anti-CAD antibody [EP710Y] - BSA and Azide free (ab231694) 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

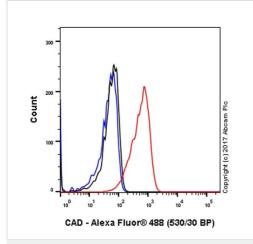
Predicted band size: 243 kDa Observed band size: 250 kDa

This data was developed using <u>**ab40800**</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

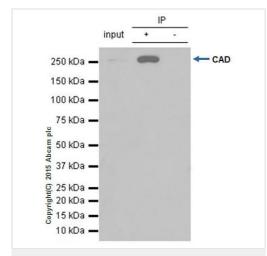
This data was developed using <u>ab40800</u>, the same antibody clone in a different buffer formulation.

Immunofluorescence staining of HeLa cells with purified <u>ab40800</u> at a working dilution of 1/300, counter-stained with DAPI. The secondary antibody was Alexa Fluor<sup>®</sup> 488 goat anti-rabbit (<u>ab150077</u>), used at a dilution of 1/1000. <u>ab7291</u>, a mouse antitubulin antibody (1/1000), was used to stain tubulin along with <u>ab150120</u> (Alexa Fluor<sup>®</sup> 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 <u>ab40800</u> was used at a dilution of 1/500 followed by an Alexa Fluor<sup>®</sup> 594 goat anti-mouse antibody (<u>ab150120</u>) at a dilution of 1/500. For negative control 2, <u>ab7291</u> (mouse antitubulin) was used at a dilution of 1/500 followed by an Alexa Fluor<sup>®</sup> 488 goat anti-rabbit antibody (<u>ab150077</u>) at a dilution of 1/400.



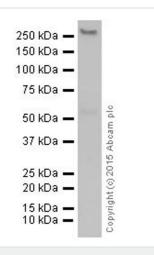
Flow Cytometry (Intracellular) - Anti-CAD antibody [EP710Y] - BSA and Azide free (ab231694) This data was developed using **<u>ab40800</u>**, the same antibody clone in a different buffer formulation.

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



This data was developed using **ab40800**, the same antibody clone in a different buffer formulation. **ab40800** (purified) at 1/30 immunoprecipitating CAD in 10 µg 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

Immunoprecipitation - Anti-CAD antibody [EP710Y] -BSA and Azide free (ab231694)



Western blot - Anti-CAD antibody [EP710Y] - BSA and Azide free (ab231694) Anti-CAD antibody [EP710Y] (**ab40800**) at 1/1000 dilution (purified) + HeLa cell lysate at 1/20000 dilution

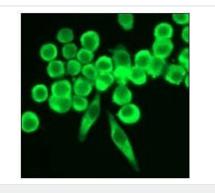
## Secondary

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

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

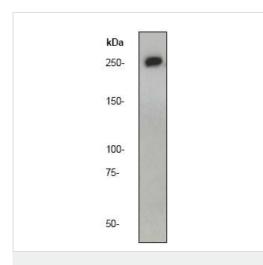
This data was developed using <u>ab40800</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



This data was developed using **<u>ab40800</u>**, the same antibody clone in a different buffer formulation.Unpurified <u>**ab40800**</u> (1/100), staining human CAD (N-term) in HeLa cells by Immunofluorescence.

Immunocytochemistry/ Immunofluorescence - Anti-CAD antibody [EP710Y] - BSA and Azide free (ab231694)



Western blot - Anti-CAD antibody [EP710Y] - BSA and Azide free (ab231694)



Anti-CAD antibody [EP710Y] - BSA and Azide free (ab231694) Anti-CAD antibody [EP710Y] (**ab40800**) at 1/1000 dilution (unpurified) **Predicted band size:** 243 kDa **Observed band size:** 250 kDa

This data was developed using <u>**ab40800**</u>, the same antibody clone in a different buffer formulation.

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