

Anti-CAD Antibody [JE33-26]

HA722248



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 243 kDa
Clone number:	JE33-26

Description: Multifunctional protein that encodes the first 3 enzymatic activities of the de novo pyrimidine pathway: carbamoylphosphate synthetase (CPSase; EC 6.3.5.5), aspartate transcarbamylase (ATCase; EC 2.1.3.2) and dihydroorotase (DHOase; EC 3.5.2.3). The CPSase-function is accomplished in 2 steps, by a glutamine-dependent amidotransferase activity (GATase) that binds and cleaves glutamine to produce ammonia, followed by an ammonium-dependent carbamoyl phosphate synthetase, which reacts with the ammonia, hydrogencarbonate and ATP to form carbamoyl phosphate. The endogenously produced carbamoyl phosphate is sequestered and channeled to the ATCase active site. ATCase then catalyzes the formation of carbamoyl-L-aspartate from L-aspartate and carbamoyl phosphate. In the last step, DHOase catalyzes the cyclization of carbamoyl aspartate to dihydroorotate.

Immunogen: Synthetic peptide within Human CAD aa 1-50 / 2,225.

Positive control: SW620 cell lysate, HeLa cell lysate, SW620, human breast cancer tissue, human tonsil tissue, HeLa.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P27708 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IHC-P	1:200
FC	1:1,000
IP	1-2µg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

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Images

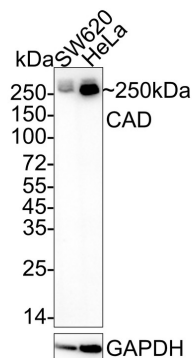


Fig1: Western blot analysis of CAD on different lysates with Rabbit anti-CAD antibody (HA722248) at 1/1,000 dilution.

Lane 1: SW620 cell lysate

Lane 2: HeLa cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 240 kDa

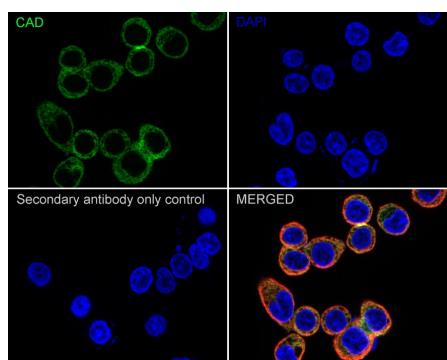
Observed band size: 250 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722248) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of SW620 cells labeling CAD with Rabbit anti-CAD antibody (HA722248) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CAD antibody (HA722248) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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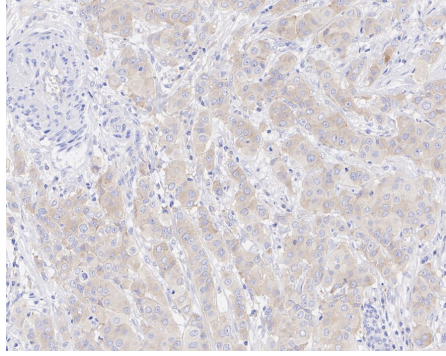


Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-CAD antibody (HA722248) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722248) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

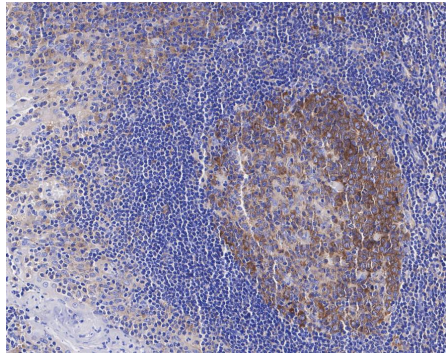


Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CAD antibody (HA722248) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722248) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

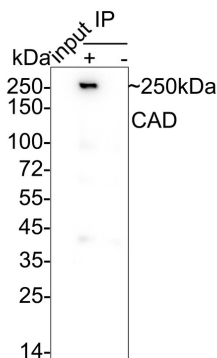


Fig5: CAD was immunoprecipitated from 0.2 mg HeLa cell lysate with HA722248 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722248 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)

Lane 2: HA722248 IP in HeLa cell lysate

Lane 3: Rabbit IgG instead of HA722248 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDN/TBST

Exposure time: 24 seconds; ECL: K1801

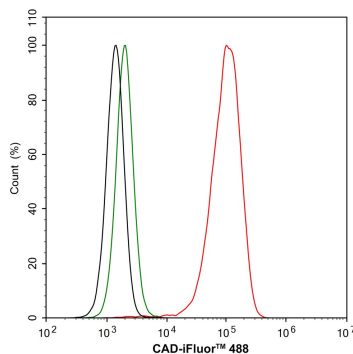


Fig6: Flow cytometric analysis of HeLa cells labeling CAD.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722248, 1 μ g/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Moreno-Morcillo M et al. Structural Insight into the Core of CAD, the Multifunctional Protein Leading De Novo Pyrimidine Biosynthesis. *Structure*. 2017 Jun
2. Nakashima A et al. Association of CAD, a multifunctional protein involved in pyrimidine synthesis, with mLST8, a component of the mTOR complexes. *J Biomed Sci*. 2013 Apr

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