abcam

Product datasheet

Anti-CTLA4 antibody [CAL49] ab237712

Recombinant RabMAb

★★★★★ <u>5 Abreviews</u> <u>15 References</u> 10 Images

Overview

Product name Anti-CTLA4 antibody [CAL49]

Description Rabbit monoclonal [CAL49] to CTLA4

Host species Rabbit

Tested applications Suitable for: IHC-P, Flow Cyt (Intra), WB, IP

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Human, Rhesus monkey

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

Positive control IHC-P: Human tonsil, lymph node and breast carcinoma tissues. WB: Human PBMCs (treated

with 10µg/ml PHA for 2 days) whole cell lysate; mouse splenocytes (treated with 2.5µg/ml Concanavalin A (ConA) for 3 days) whole cell lysate. Flow Cyt (intra): Human PBMCs (treated with 10µg/ml PHA for 2 days); mouse splenocytes (treated with 2.5µg/ml Concanavalin A

(ConA) for 3 days). IP: Human tonsil lysate.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.05% Sodium azide

Constituent: PBS

Purity Protein A purified

Purification notes Purity >99%.

Clonality Monoclonal

Clone number CAL49

Isotype IgG

Applications

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The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab237712 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
IHC-P	*** *	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/400.
WB		1/1000. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa).
IP		1/30.

Application notes

Is unsuitable for ICC/IF.

Target

Function

Inhibitory receptor acting as a major negative regulator of T-cell responses. The affinity of CTLA4 for its natural B7 family ligands, CD80 and CD86, is considerably stronger than the affinity of their cognate stimulatory coreceptor CD28.

Tissue specificity

Widely expressed with highest levels in lymphoid tissues. Detected in activated T-cells where expression levels are 30- to 50-fold less than CD28, the stimulatory coreceptor, on the cell surface following activation.

Involvement in disease

Genetic variation in CTLA4 influences susceptibility to systemic lupus erythematosus (SLE) [MIM:152700]. SLE is a chronic, inflammatory and often febrile multisystemic disorder of connective tissue. It affects principally the skin, joints, kidneys and serosal membranes. SLE is thought to represent a failure of the regulatory mechanisms of the autoimmune system. Note=Genetic variations in CTLA4 may influence susceptibility to Graves disease, an autoimmune disorder associated with overactivity of the thyroid gland and hyperthyroidism. Genetic variation in CTLA4 is the cause of susceptibility to diabetes mellitus insulin-dependent type 12 (IDDM12) [MIM:601388]. A multifactorial disorder of glucose homeostasis that is characterized by susceptibility to ketoacidosis in the absence of insulin therapy. Clinical fetaures are polydipsia, polyphagia and polyuria which result from hyperglycemia-induced osmotic diuresis and secondary thirst. These derangements result in long-term complications that affect the eyes, kidneys, nerves, and blood vessels.

Genetic variation in CTLA4 is the cause of susceptibility to celiac disease type 3 (CELIAC3) [MIM:609755]. It is a multifactorial disorder of the small intestine that is influenced by both environmental and genetic factors. It is characterized by malabsorption resulting from inflammatory injury to the mucosa of the small intestine after the ingestion of wheat gluten or related rye and barley proteins. In its classic form, celiac disease is characterized in children by malabsorption and failure to thrive.

Sequence similarities

Contains 1 lg-like V-type (immunoglobulin-like) domain.

Post-translational modifications

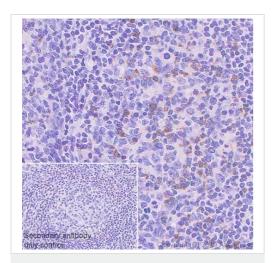
N-glycosylation is important for dimerization.

Phosphorylation at Tyr-201 prevents binding to the AP-2 adapter complex, blocks endocytosis,

and leads to retention of CTLA4 on the cell surface.

Cellular localization

Cell membrane. Exists primarily an intracellular antigen whose surface expression is tightly regulated by restricted trafficking to the cell surface and rapid internalisation and.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTLA4 antibody [CAL49] (ab237712)

(ab237712)

1 2 3 4

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
37 kDa —
38 kW 25 kDa —
48 kW 25 kDa —
49 kW 25 kDa —
40 kDa —

GAPDH

Western blot - Anti-CTLA4 antibody [CAL49] (ab237712)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CTLA4 with ab237712 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on human tonsil is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins

The section was incubated with ab237712 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

All lanes : Anti-CTLA4 antibody [CAL49] (ab237712) at 1/1000 dilution

Lane 1 : Untreated human PBMC (human peripheral blood mononuclear cell) whole cell lysate

Lane 2 : Human PBMC (treated with $10\mu g/ml$ PHA for 2 days) whole cell lysate

Lane 3: Untreated mouse splenocyte whole cell lysate

Lane 4: Mouse splenocyte (treated with 2.5µg/ml Concanavalin A (ConA) for 3 days) whole cell lysate

Lysates/proteins at 20 µg per lane.

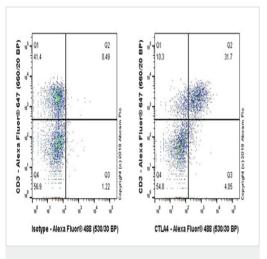
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

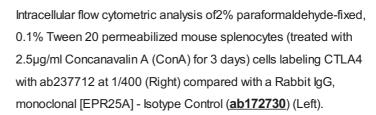
Predicted band size: 25 kDa Observed band size: 25 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

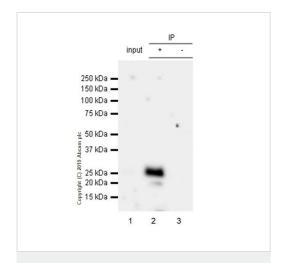


Flow Cytometry (Intracellular) - Anti-CTLA4 antibody [CAL49] (ab237712)



Cells were surface stained with anti-CD3 conjugated to Alexa Fluor $^{(\!0\!)}$ 647. Then fixed with 2% PFA for 10min followed by intracellular staining with rabbit lgG (Left) and ab237712 (Right).

Goat Anti-Rabbit IgG Fc (Alexa Fluor[®] 488) preadsorbed (<u>ab150097</u>), at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CTLA4 antibody [CAL49] (ab237712)

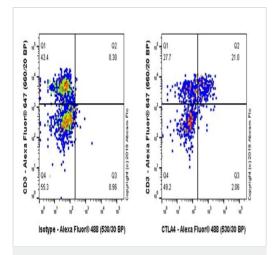
CTLA4 was immunoprecipitated from 0.35 mh human tonsil lysate with ab237712 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab237712 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/1000 dilution.

Lane 1: Human tonsil lysate 10 µg (Input).

Lane 2: ab237712 IP in human tonsil lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab237712 in human tonsil lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 30 seconds.

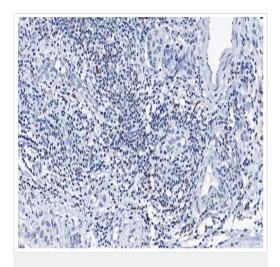


Flow Cytometry (Intracellular) - Anti-CTLA4 antibody [CAL49] (ab237712)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween 20 permeabilized human PBMC (peripheral blood mononuclear cell) (treated with 10µg/ml PHA for 2 days) cells labeling CTLA4 with ab237712 at 1/400 (Right) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Left).

Cells were surface stained with anti-CD3 conjugated to Alexa Fluor $^{\tiny{(8)}}$ 647. Then fixed with 2% PFA for 10min followed by intracellular staining with rabbit lgG (Left) and ab237712 (Right).

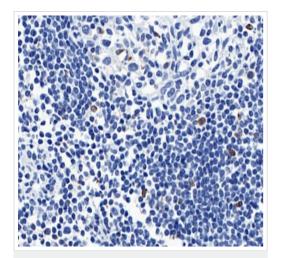
Goat Anti-Rabbit IgG Fc (Alexa Fluor[®] 488) preadsorbed (<u>ab150097</u>), at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTLA4 antibody [CAL49] (ab237712)

Formalin-fixed, paraffin-embedded human breast carcinoma tissue stained for CTLA4 using ab237712 at 0.25 μ g/ml in immunohistochemical analysis.

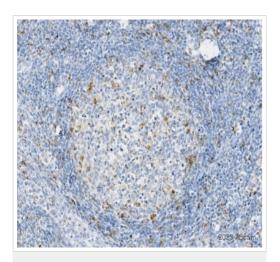
Incubate with primary antibody for 75 minutes at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTLA4 antibody [CAL49] (ab237712)

Formalin-fixed, paraffin-embedded human lymph node tissue stained for CTLA4 using ab237712 at 0.25 μ g/ml in immunohistochemical analysis.

Incubate with primary antibody for 75 minutes at room temperature.



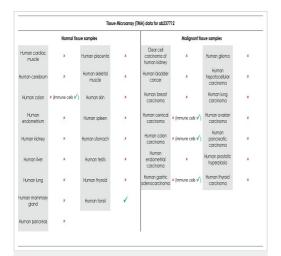
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTLA4 antibody [CAL49] (ab237712)

This image is courtesy of Dr. Chi Ngai Chan

Immunohistochemical analysis of paraffin-embeded Rhesus monkey tonsil tissue labeling CTLA4 with <u>ab251599</u> followed by Polink 1 Polymer HRP anti-Rabbit lgG.

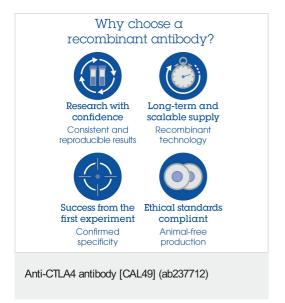
Heat mediated antigen retrieval-Buffer/Enzyme Used: Dako pH9.

This data was developed using the same antibody clone in a different buffer formulation (ab251599).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTLA4 antibody [CAL49] (ab237712)

Tissue Microarrays stained for "Anti-CTLA4 antibody [CAL49]" using "ab237712" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond TM Epitope Retrieval Solution 2 (pH 9.0) for 30 minutes. The sections were incubated with ab237712 for 30 mins at room temperature followed by a ready to use LeicaDS9800 (Bond $^{\&}$ Polymer Refine Detection). The immunostaining was performed on a Leica Biosystems BOND $^{\&}$ RX instrument.



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