abcam

Product datasheet

Anti-CD3 epsilon antibody [CAL57] - BSA and Azide free ab251607

Recombinant RabMAb

10 Images

Overview

Product name Anti-CD3 epsilon antibody [CAL57] - BSA and Azide free

Description Rabbit monoclonal [CAL57] to CD3 epsilon - BSA and Azide free

Host species Rabbit

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

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide within Mouse CD3 epsilon aa 135-189. The exact sequence is proprietary.

Database link: P22646

Positive control IHC-P: Mouse and rat spleen tissue. Human tonsil and colon tissue. WB: EL4.IL-2 and Jurkat

> whole cell lysate. Mouse lymph node lysate. Rat thymus and spleen lysate. IHC-Fr: Mouse thymus tissue. Flow Cyt (intra): Human peripheral blood mononuclear cells. Mouse splenocytes. IP:

EL4.IL-2 whole cell lysate.

General notes ab251607 is the carrier-free version of ab237721.

> Our carrier-free 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 cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

Properties

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

Purification notes Purity is greater than 99%.

Clonality Monoclonal

Clone number CAL57

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab251607 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 23 kDa.
IHC-Fr		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

Target

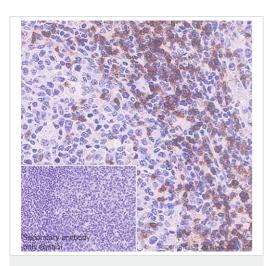
Function The CD3 complex mediates signal transduction.

Sequence similaritiesContains 1 lg-like (immunoglobulin-like) domain.

Contains 1 ITAM domain.

Cellular localization Membrane.

Images

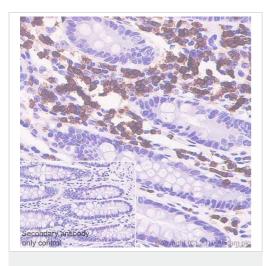


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody

[CAL57] - BSA and Azide free (ab251607)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the human tonsil. The section was incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

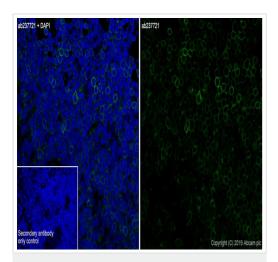
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237721).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody

[CAL57] - BSA and Azide free (ab251607)

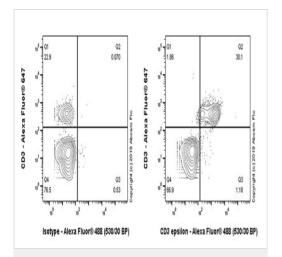
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling CD3 epsilon with <u>ab237721</u> at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining on the T lymphocytes in the human colon. The section was incubated with <u>ab237721</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Immunohistochemistry (Frozen sections) - Anti-CD3 epsilon antibody [CAL57] - BSA and Azide free (ab251607)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse thymus tissue labeling CD3 epsilon with ab237721 at 1/500 dilution (green), followed by ab150077 AlexaFluor[®]488 Goat anti-Rabbit secondary at a 1/1,000 dilution. Positive staining on T cell surface (PMID: 8490660) is observed. Counterstained with DAPI (blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 AlexaFluor[®]488 Goat anti-Rabbit used at a 1/1,000 dilution. Heat mediated antigen retrieval using sodium citrate buffer (10 mM citrate pH 6.0 and 0.05% Tween-20).

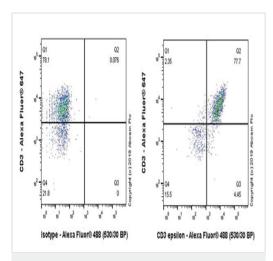
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237721).



Flow Cytometry (Intracellular) - Anti-CD3 epsilon antibody [CAL57] - BSA and Azide free (ab251607)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized mouse splenocytes labeling CD3 epsilon with ab237721 at 1/500 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor[®] 488, ab150077), at 1/2000 dilution was used as the secondary antibody.

Cells were surface stained with anti-CD3 conjugated to Alexa Fluor[®] 647. Then fixed with 2% PFA for 10min and intracellularly stained with rabbit IgG (Left) or <u>ab237721</u> (Right). They recognize the same cell population.

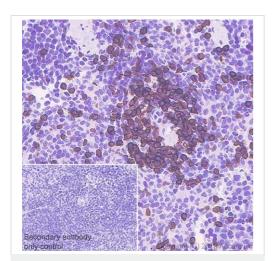


Flow Cytometry (Intracellular) - Anti-CD3 epsilon antibody [CAL57] - BSA and Azide free (ab251607)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized human peripheral blood mononuclear cell (PBMC) labeling CD3 epsilon with ab237721 at 1/500 (right panel) compared with a Rabbit monoclonal IgG (ab172730) (left panel). Goat anti rabbit IgG (Alexa Fluor[®] 488, ab150097), at 1/5000 dilution was used as the secondary antibody.

Cells were surface stained with anti-CD3 conjugated to Alexa Fluor[®] 647. Then fixed with 2% PFA for 10min and intracellularly stained with rabbit lgG (Left) or <u>ab237721</u> (Right). They recognize the same cell population.

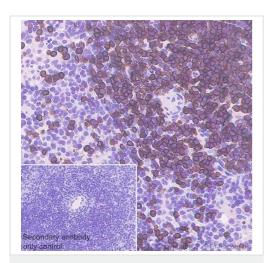
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237721).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody

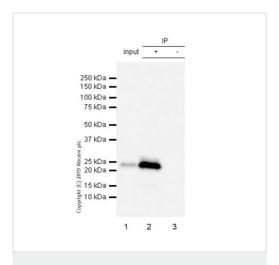
[CAL57] - BSA and Azide free (ab251607)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the rat spleen. The section was incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody

[CAL57] - BSA and Azide free (ab251607)



Immunoprecipitation - Anti-CD3 epsilon antibody

[CAL57] - BSA and Azide free (ab251607)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD3 epsilon with ab237721 at 1/2000 dilution, followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the T lymphocytes in the mouse spleen. The section was incubated with ab237721 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237721).

CD3 epsilon was immunoprecipitated from 0.35 mg EL4.IL2 (Mouse lymphoma T lymphocyte) whole cell lysate with <u>ab237721</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab237721</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used at 1/5000 dilution.

Lane 1: EL4.IL2 whole cell lysate 10 µg (Input).

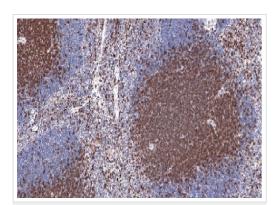
Lane 2: ab237721 IP in EL4.IL2 whole cell lysate.

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of $\underline{ab237721}$ in

EL4.IL2 whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 5 seconds.

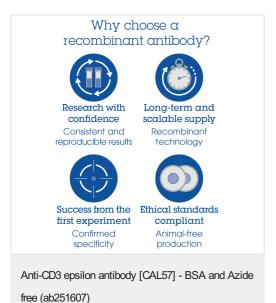


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody

[CAL57] - BSA and Azide free (ab251607)

Formalin-fixed, paraffin-embedded mouse spleen tissue stained for CD3 epsilon using <u>ab237721</u> at $0.5~\mu g/ml$ in immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237721).



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