abcam

ab251594

Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	CAL54
Purification technique	Affinity purification Protein A
Concentration	1.038 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purity is greater than 99%.

Reactivity data

mIHC

Tested

Species	Human

Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information CD3E

Function	Part of the TCR-CD3 complex present on T-lymphocyte cell surface that plays an essential role in adaptive immune response (PubMed:15294938, PubMed:15546002, PubMed:2470098, PubMed:40592325, PubMed:8490660). When antigen presenting cells (APCs) activate T-cell receptor (TCR), TCR-mediated signals are transmitted across the cell membrane by the CD3 chains CD3D, CD3E, CD3G and CD247/CD3Z (PubMed:2470098, PubMed:40592325). All CD3 chains contain immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domain (PubMed:2470098, PubMed:40592325). Upon TCR engagement, these motifs become phosphorylated by Src family protein tyrosine kinases LCK and FYN, resulting in the activation of downstream signaling pathways (PubMed:2470098, PubMed:40592325). CD3E ITAM phosphorylation creates docking sites for the protein kinase ZAP70 leading to ZAP70 phosphorylation and its conversion into a catalytically active enzyme (By similarity). In addition of this role of signal transduction in T-cell activation, CD3E plays an essential role in correct T-cell development (By similarity). Also participates in internalization and cell surface down-regulation of TCR-CD3 complexes via endocytosis sequences present in CD3E cytosolic region (PubMed:10384095, PubMed:26507128). In addition to its role as a TCR coreceptor, it serves as a receptor for ITPRIPL1 (PubMed:38614099). Ligand recognition inhibits T-cell activation by promoting interaction with NCK1, which prevents CD3E-ZAP70 interaction and blocks the ERK-NFkB signaling cascade and calcium influx (PubMed:12110186, PubMed:38614099).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab251594 is the carrier-free version of ab237707.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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 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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

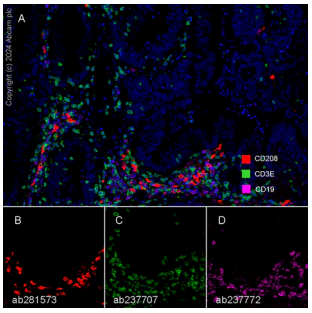
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

13 product images



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

This data was developed using ab237707, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon cancer labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human colon cancer.

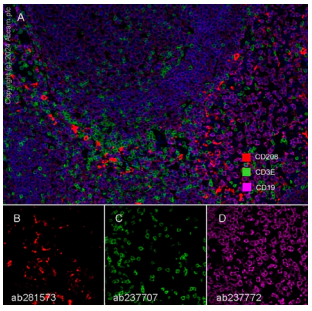
Panel B: anti-CD208 staining dendritic cells in human colon cancer.

Panel C: anti-CD3E staining T lymphocytes in human colon cancer.

Panel D: anti-CD19 staining B lymphocytes of human colon cancer.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Multiplex immunohistochemistry staining of Human tonsil tissue using rabbit Anti-CD3 epsilon antibody

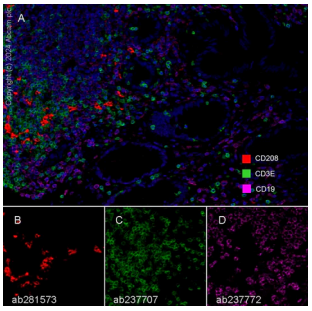
This data was developed using ab237707, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human tonsil.
Panel B: anti-CD208 staining dendritic cells in human tonsil.
Panel C: anti-CD3E staining T lymphocytes in human tonsil.
Panel D: anti-CD19 staining B lymphocytes of human tonsil.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

This data was developed using ab237707, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human colon.

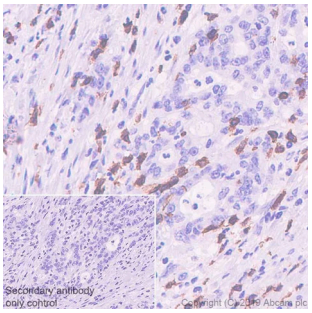
Panel B: anti-CD208 staining dendritic cells in human colon.

Panel C: anti-CD3E staining T lymphocytes in human colon.

Panel D: anti-CD19 staining B lymphocytes of human colon.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

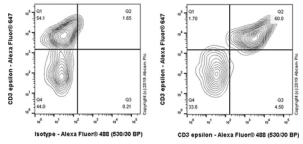
Immunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue labeling CD3 epsilon with ab237707 at 1/500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the infiltrating T lymphocytes in the human gastric carcinoma is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab237707 for 30 mins at room temperature.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).

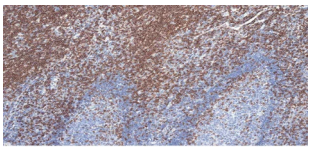


Flow Cytometry (Intracellular) - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Flow Cytometry (Intracellular) staining using rabbit Anti-CD3 epsilon antibody

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% Tween 20 permeabilized human PBMCs (peripheral blood mononuclear cells) labeling CD3 epsilon with ab237707 at 1/500 (Right compared with a Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Left). Goat Anti-Rabbit IgG Fc (Alexa Fluor® 488) preadsorbed (ab150097), at 1/5000 dilution was used as the secondary antibody.
Cells were surface stained with anti-CD3 epsilon conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min followed by intracellular staining rabbit IgG (Left) or ab237707 (Right).

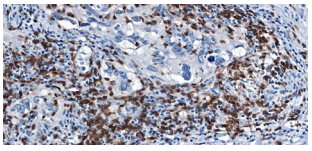
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

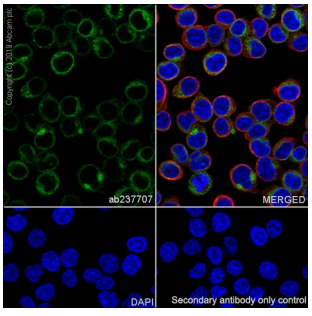
Formalin-fixed, paraffin-embedded human tonsil tissue stained for CD3 epsilon using ab237707 at 0.5 µg/mL in immunohistochemcial analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

Formalin-fixed, paraffin-embedded human NSCLC tissue stained for CD3 epsilon using ab237707 at 0.5 µg/mL in immunohistochemcial analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).

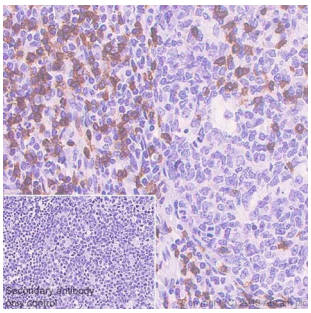


Immunocytochemistry/ Immunofluorescence - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD3 epsilon antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (human T cell leukemia cell line from peripheral blood) cells labeling CD3 epsilon with ab237707 at 1/55 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in Jurkat cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).
PBS only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD3 epsilon antibody

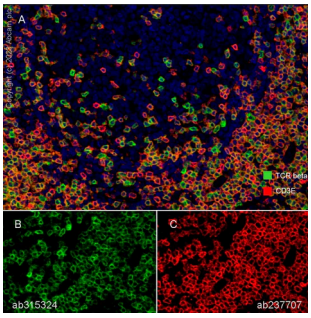
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD3 epsilon with ab237707 at 1/500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the human tonsil is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab237707 for 30 mins at room temperature.

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

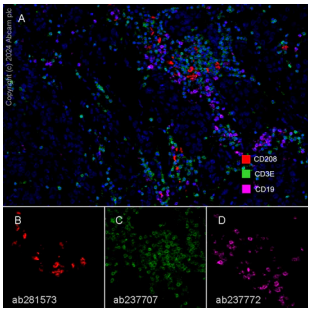
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

This data was developed using ab237707, the same antibody clone in a different buffer formulation.

Fluorescence multiplex immunohistochemical analysis of human tonsil (formalin-fixed paraffin-embedded section). Merged staining of Panel A: merged staining of anti-TCR beta (green; Opal™520) and anti-CD3E (red; Opal™570) on human tonsil, Panel B: anti-TCR beta staining T lymphocytes of human tonsil, Panel C: anti-CD3E staining T lymphocytes of human tonsil. DAPI was used as a counter stain. Followed by Opal Polymer HRP Ms + Rb. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. The section was incubated in three rounds of staining: in the order of ab315324, ab237707 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

This data was developed using ab237707, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human liver cancer labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary

antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human liver cancer.

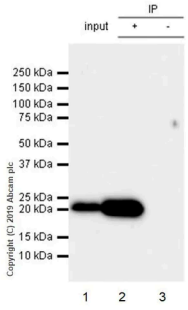
Panel B: anti-CD208 staining dendritic cells in human liver cancer.

Panel C: anti-CD3E staining T lymphocytes in human liver cancer.

Panel D: anti-CD19 staining B lymphocytes of human liver cancer.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Immunoprecipitation - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon was immunoprecipitated from 0.35 mg of Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate with ab237707 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab237707 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.
Lane 1: Jurkat whole cell lysate 10 µg (Input).
Lane 2: ab237707 IP in Jurkat whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237707 in Jurkat whole cell lysate.

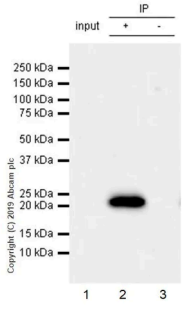
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).

All lanes:
Immunoprecipitation - Anti-CD3 epsilon antibody [CAL54] (ab237707)

Predicted band size: 23 kDa

Observed band size: 21 kDa



Immunoprecipitation - Anti-CD3 epsilon antibody [CAL54] - BSA and Azide free (ab251594)

CD3 epsilon was immunoprecipitated from 0.35 mg of human thymus lysate with ab237707 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab237707 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.
Lane 1: Human thymus lysate 10 µg (Input).
Lane 2: ab237707 IP in human thymus lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237707 in human thymus lysate.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab237707).
All lanes:
Immunoprecipitation - Anti-CD3 epsilon antibody [CAL54] (ab237707)

Predicted band size: 23 kDa

Observed band size: 21 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.