abcam

## Product datasheet

# Anti-GITR antibody [CAL52] - BSA and Azide free ab251600

Recombinant

6 Images

### Overview

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|---|---|
| **Product name** | Anti-GITR antibody [CAL52] - BSA and Azide free |
| **Description** | Rabbit monoclonal [CAL52] to GITR - BSA and Azide free |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** IHC-P, ICC/IF, IP, Flow Cyt<br>**Unsuitable for:** WB |
| **Species reactivity** | **Reacts with:** Human |
| **Immunogen** | Synthetic peptide corresponding to Human GITR aa 150-250.<br>Database link: Q9Y5U5 |
| **Positive control** | IHC-P: Human tonsil tissue. ICC/IF: HEK-293T cells. IP: HEK-293T and Hut-78 whole cell lysate. Flow: Human peripheral blood mononuclear cells. |
| **General notes** | ab251600 is a PBS-only buffer format of ab237713. Please refer to ab237713 for recommended dilutions, protocols, and image data.<br><br>The formulation and the concentration of this product is compatible for metal-conjugation for mass cytometry (CyTOF®).<br><br>*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.*<br><br>This product was previously labelled as TNFRSF18 |

### Properties

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| **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. |
| **Purity** | Protein A purified |

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| **Purification notes** | Purity is greater than 99%. |
| **Clonality** | Monoclonal |
| **Clone number** | CAL52 |
| **Isotype** | IgG |

## Applications

Our Abpromise guarantee covers the use of **ab251600** 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 | | Use at an assay dependent concentration.<br>Boil tissue section in TRIS EDTA buffer for 24 min followed by cooling at room temperature for 30-45 min.<br>Primary antibody incubation for 75 minutes at room temperature. |
| ICC/IF | | Use at an assay dependent concentration. |
| IP | | Use at an assay dependent concentration. |
| Flow Cyt | | Use at an assay dependent concentration. |

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| **Application notes** | Is unsuitable for WB. |

## Target

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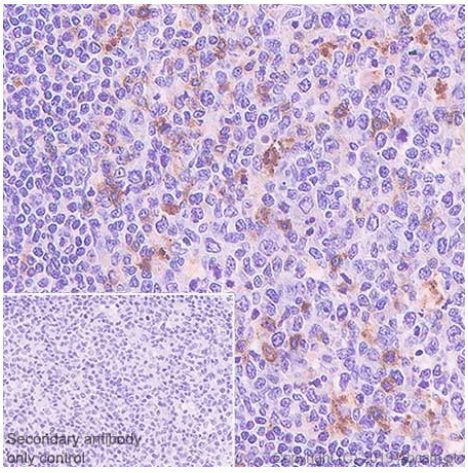
| **Function** | Receptor for TNFSF18. Seems to be involved in interactions between activated T-lymphocytes and endothelial cells and in the regulation of T-cell receptor-mediated cell death. Mediated NF-kappa-B activation via the TRAF2/NIK pathway. |
| **Tissue specificity** | Expressed in lymph node, peripheral blood leukocytes and weakly in spleen. |
| **Sequence similarities** | Contains 3 TNFR-Cys repeats. |
| **Cellular localization** | Secreted and Cell membrane. |

## Images

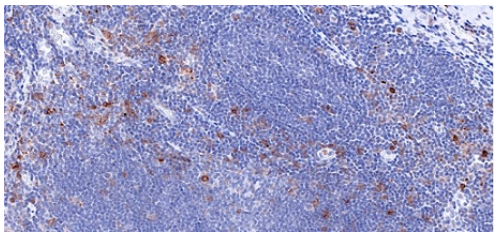



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling GITR with ab237713 at 1/4000 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on the human tonsil is observed. Counter stained with Hematoxylin. The section was incubated with ab237713 for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

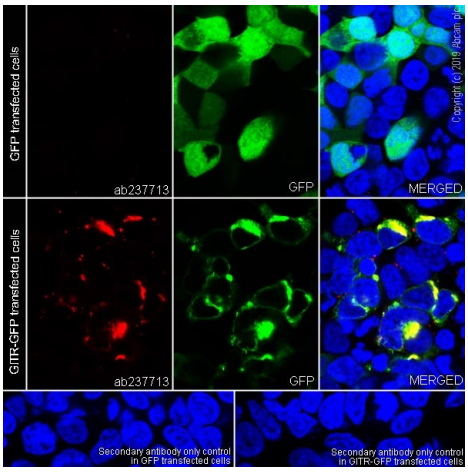
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

Formalin-fixed, paraffin-embedded human tonsil tissue stained for TNFSF18 using ab237713 at 0.25 µg/ml in immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).

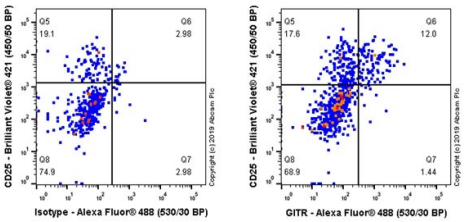



Immunocytochemistry/ Immunofluorescence - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

4% Paraformaldehyde-fixed 0.1% TritonX-100 permeabilized HEK-293T (human embryonic kidney epithelial cell) cells labeling GITR with ab237713 at 1/50 dilution followed by a AlexaFluor®594 Goat anti-Rabbit secondary (ab150080) at a 1/500 dilution (Green). The nuclear counterstain was DAPI (Blue). Confocal image showing Positive staining in HEK-293T cells transfected with a GFP-tagged GITR expression construct.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is an AlexaFluor®594 Goat anti-Rabbit secondary (ab150080) at a 1/500 dilution.

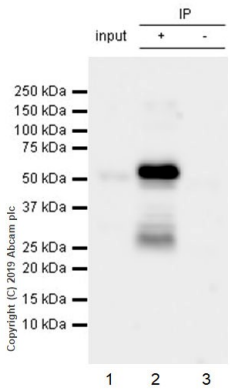
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).




Flow Cytometry - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

Flow cytometric analysis of 2% paraformaldehyde-fixed, 0.1% tween-20 permeabilized Human peripheral blood mononuclear cell (PBMC) treated with 10µg/ml PHA for 48h, labeling GITR with ab237713 at 1/500 dilution. The secondary antibody was a Goat anti rabbit IgG (Alexa Fluor® 488, ab150097) at 1/500 dilution. Cells were surface stained with anti-CD25 conjugated to BV421. Then fixed with 2% PFA followed by intracellular staining rabbit IgG (Left) or ab237713 (Right). The isotype control used was a Rabbit monoclonal IgG (ab172730, Left).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).




Immunoprecipitation - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

GITR was immunoprecipitated from 0.35 mg Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate using ab237713 at 1/30 dilution. western blot was performed on the immunoprecipitate using ab237713 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used as the secondary antibody at 1/5000 dilution.

Lane 1: Hut-78 whole cell lysate 10 µg (input)
Lane 2: ab237713 IP in Hut-78 whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237713 in Hut-78 whole cell lysate.

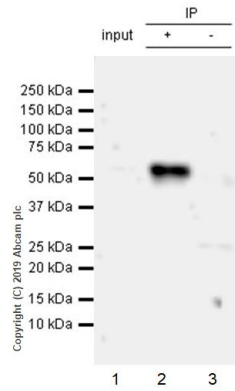
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds.

This blot was developed using a higher sensitivity ECL substrate.

Dimerized GITR was also observed at 52kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).




Immunoprecipitation - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

GITR was immunoprecipitated from 0.35 mg HEK-293T (Human embryonic kidney epithelial cell) transfected with GFP-tagged GITR overexpression vector whole cell lysate using ab237713 at 1/30 dilution. western blot was performed on the immunoprecipitate using ab237713 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used as the secondary antibody at 1/5000 dilution.

Lane 1: Hut-78 whole cell lysate 10 µg (input)
Lane 2: ab237713 IP in Hut-78 whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab237713 in 293T transfected with GFP-tagged GITR overexpression vector whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

This blot was developed using a higher sensitivity ECL substrate.

Dimerized GITR was also observed at 52kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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