# abcam

## Product datasheet

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



## 7 Images

#### Overview

**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, Flow Cyt (Intra), ICC/IF, IP

Unsuitable for: WB

Species reactivity Reacts with: Human

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

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 the carrier-free version of ab237713.

> 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 CAL52

**Isotype** IgG

### **Applications**

The Abpromise guarantee 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.  Boil tissue section in TRIS EDTA buffer for 24 min followed by cooling at room temperature for 30-45 min. Primary antibody incubation for 75 minutes at room temperature.
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

**Application notes** Is unsuitable for WB.

**Target** 

**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-

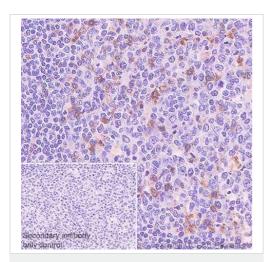
kappa-B activation via the TRAF2/NIK pathway.

**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

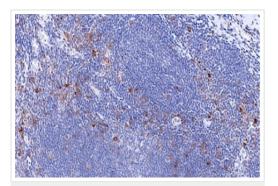


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

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

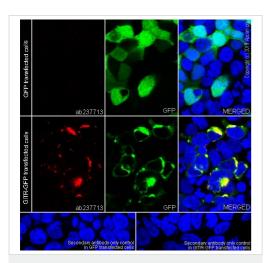
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).



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

Formalin-fixed, paraffin-embedded human tonsil tissue stained for TNFSF18 using <u>ab237713</u> at  $0.25~\mu g/ml$  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).

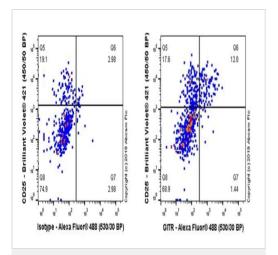


Immunocytochemistry/ Immunofluorescence - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

4% Paraformaldehyde-fixed 0.1% TritonX-100 permeabilized HEK-293T (human embryonic kidney epithelial cell) cells labeling GITR with <u>ab237713</u> at 1/50 dilution followed by a AlexaFluor<sup>®</sup>594 Goat anti-Rabbit secondary (<u>ab150080</u>) 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<sup>®</sup>594 Goat anti-Rabbit secondary (<u>ab150080</u>) at a 1/500 dilution.

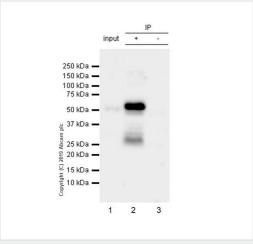
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab237713).

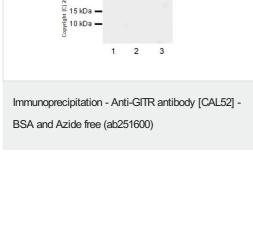


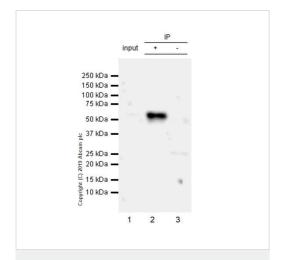
Flow Cytometry (Intracellular) - Anti-GITR antibody [CAL52] - BSA and Azide free (ab251600)

Intracellular 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 GITRwith <a href="mailto:ab237713">ab237713</a> at 1/500 dilution. The secondary antibody was a Goat anti rabbit lgG (Alexa Fluor 488, <a href="mailto:ab150097">ab150097</a>) at 1/500 dilution. Cells were surface stained with anti-CD25 conjugated to BV421. Then fixed with 2% PFA followed by intracellular staining rabbit lgG (Left) or <a href="mailto:ab237713">ab237713</a> (Right). The isotype control used was a Rabbit monoclonal lgG (<a href="mailto:ab172730">ab172730</a>, 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 <a href="mailto:ab237713">ab237713</a> at 1/30 dilution. western blot was performed on the immunoprecipitate using <a href="mailto:ab237713">ab237713</a> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<a href="mailto:ab131366">ab131366</a>) 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  $\lg G$  ( $\underline{ab172730}$ ) instead of  $\underline{ab237713}$  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).

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 <u>ab237713</u> at 1/30 dilution. western blot was performed on the immunoprecipitate using <u>ab237713</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) 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 (<u>ab172730</u>) instead of <u>ab237713</u> in 293T transfected with GFP-tagged GITR overexpression vector whole cell lysate

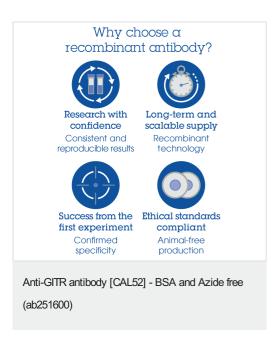
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

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).



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