Product name: Anti-PD1 antibody [NAT105] ab52587

Description: Mouse monoclonal [NAT105] to PD1

Host species: Mouse

Specificity: This antibody recognizes human PD1, a checkpoint protein expressed by activated T and B cells. PD1 is involved in the control of immune cell responses.

Tested applications: Suitable for: WB, Flow Cyt, IHC-Fr, ICC/IF, IP, IHC-P

Species reactivity: Reacts with: Human

Immunogen: Tissue, cells or virus corresponding to Human PD1. Specifically, human YT cells (NK-like leukemia cell line) that express PD1.

Database link: Q15116


General notes: Please note that PD-1 is expressed variably in different tissues and that optimisation may be required depending on the tissue used for the experiment.

Western blot protocol advice:
Due to low expression of PD-1, we recommend loading a high amount of sample (100 µg) to detect the band for PD-1. Human tonsil and YT cell line lysates are suitable positive controls.

This antibody clone [NAT105] is manufactured by Abcam. If you require a different buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com.

New alternative versions of the NAT105 clone available:
Recombinant version (ab234444)
PBS-only recombinant version (ab201811)
Chimeric recombinant rabbit version (ab216352)

Form: Liquid

Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer: pH: 7.40
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine

Purity
Protein G purified

Clonality
Monoclonal

Clone number
NAT105

Isotype
IgG1

Light chain type
kappa

Applications

Our Abpromise guarantee covers the use of ab52587 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>1/50. Predicted molecular weight: 32 kDa.</td>
<td></td>
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<tr>
<td>Flow Cyt</td>
<td>1/100. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IHC-Fr</td>
<td>★★★★☆ Use at an assay dependent concentration.</td>
<td></td>
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<tr>
<td>ICC/IF</td>
<td>Use a concentration of 5 - 10 µg/ml. We recommend Goat Anti-Mouse IgG H&amp;L (Alexa Fluor® 488) (ab150117) secondary antibody.</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>Use at an assay dependent concentration.</td>
<td></td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★ 1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
<td></td>
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</tbody>
</table>

Target

Function
Possible cell death inducer, in association with other factors.

Involvement in disease
Genetic variation in PDCD1 is associated with susceptibility to systemic lupus erythematosus type 2 (SLEB2) [MIM:605218]. Systemic lupus erythematosus is a chronic, inflammatory and often febrile multisystemic disorder of connective tissue. It affects principally the skin, joints, kidneys and serosal membranes. It is thought to represent a failure of the regulatory mechanisms of the autoimmune system.

Sequence similarities
Contains 1 Ig-like V-type (immunoglobulin-like) domain.

Developmental stage
Induced at programmed cell death.

Cellular localization
Membrane.

Images
IHC image of PD1 staining in normal human tonsil formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab52587 at 5 µg/ml for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with hematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

Double immunofluorescence staining of CD3 (green) and PD1 (red) on paraffin embedded tonsil.

Sample: Human tonsil cell extract
Dilution: ab52587 antibody was used as 1/200 in 1x10^6 cells/tube.
Anti-CD4 antibody was used as 1/200 dilution
Anti-CD8 antibody was used as 1/200 dilution
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [NAT105] (ab52587)

Image courtesy of an AbReview submitted by Dr Hajnalia Rajnai

Formaldehyde-fixed, paraffin-embedded human follicular lymphoma tissue stained for PD1 with ab52587 at 1/100 dilution in immunohistochemical analysis.

Western blot - Anti-PD1 antibody [NAT105] (ab52587)

Anti-PD1 antibody [NAT105] (ab52587) at 1/50 dilution + YT cell line extracts

**Predicted band size:** 32 kDa

**Observed band size:** 47 kDa

why is the actual band size different from the predicted?
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [NAT105] (ab52587)


Fig 1. Representative immunohistochemical staining results for PD1 using ab52587 at a 1/50 dilution in human formalin-fixed, paraffin-embedded tissue specimens.

Panel A: Normal lung tissue, negative control;
Panel B: Tonsillar tissue, positive control;
Panel C: PD1-negative tumor infiltrating lymphocytes;
Panel D: PD1-positive tumor infiltrating lymphocytes in squamous cell carcinomas.

Immunohistochemical analysis of various soft tissue sarcomas staining PD1 using ab52587 at a 1/50 dilution.
Arrows indicate PD1 positive lymphocytes.

Overlay histogram showing MOLT-4 (Human lymphoblastic leukemia cell line) cells stained with ab52587 (red line). Live cells were incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab52587, 1/100 dilution) for 30 min at 4°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150117) at 1/2000 dilution for 30 min at 4°C. A mouse IgG1 isotype control antibody (ab170190) was used at the same concentration and conditions as the primary antibody (black line). Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.
MOLT-4 (Human lymphoblastic leukemia cell line) cells stained for PD1 (colored green) using ab52587 in ICC/IF.

Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% Tween-20 for 1 hour at room temperature to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with ab52587 at 10 µg/ml and ab6046 (Rabbit polyclonal to beta Tubulin - Loading Control) at 1 µg/ml overnight at +4°C. The secondary antibodies used were Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (ab150117) secondary antibody used at 1 µg/ml (colored green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (ab150080) secondary antibody (pseudo-colored red) used at 2 µg/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.

Immunohistochemical analysis of human large and locally advanced breast cancers staining PD1 using ab52587.

(Panel a) Low level of PD-1^+ T cell infiltration.
(Panel b) high level of PD-1^+ T cell infiltration. (Itu: intratumoral Str: stromal).
Double immunoenzymatic staining of Ki67 (brown) and PD1 (red) on paraffin embedded tonsil.

Human tonsil tissue stained for PD1 with ab52587 incubated for 30 mins at a 1/100 dilution in immunohistochemical analysis.

Immunohistochemical analysis of frozen human liver tissue labeling PD1 with ab52587 at 1/50 dilution.
Expression of markers of T cell differentiation and degree of inflammation in the heart of chronically T. cruzi-infected subjects with severe cardiomyopathy.

A–D, left panel: representative photos of CD45RO, CD27, PD1 and CD57 expression, respectively.

Panel C shown.

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