Anti-PYK2 antibody [YE353] ab32571

Overview

Product name
Anti-PYK2 antibody [YE353]

Description
Rabbit monoclonal [YE353] to PYK2

Host species
Rabbit

Specificity
This antibody recognizes PYK2. It does not cross react with other FAK family members.

Tested applications
Suitable for: WB, IHC-P, ICC/IF
Unsuitable for: IP

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide within Human PYK2 aa 1-100 (N terminal). The exact sequence is proprietary.
Database link: Q14289

Positive control
WB: Ramos, Jurkat and RAW264.7 cell lysates and mouse and rat brain tissue lysates. IHC-P: Human, mouse and rat cerebral cortex tissues. ICC/IF: HeLa and PC12 cells.

General notes

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form
Liquid

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

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
YE353

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab32571 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Abreviews</th>
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<tr>
<td>WB</td>
<td>1/2000</td>
<td>Detects a band of approximately 116 kDa (predicted molecular weight: 116 kDa). For unpurified use at 1/1000 - 1/5000.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>1/300</td>
<td>See IHC antigen retrieval protocols. For unpurified use at 1/250 - 1/500.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>1/60</td>
<td>For unpurified use at 1/100.</td>
</tr>
</tbody>
</table>

Application notes
Is unsuitable for IP.

Target

Function
Involved in calcium induced regulation of ion channel and activation of the map kinase signaling pathway. May represent an important signaling intermediate between neuropeptide activated receptors or neurotransmitters that increase calcium flux and the downstream signals that regulate neuronal activity. Interacts with the SH2 domain of Grb2. May phosphorylate the voltage-gated potassium channel protein Kv1.2. Its activation is highly correlated with the stimulation of c-Jun N-terminal kinase activity. Involved in osmotic stress-dependent SNCA 'Tyr-125' phosphorylation. In concert with SRC, plays an important role in osteoclastic bone resorption. Both the formation of a SRC-PTK2B complex, and SRC kinase activity are necessary for this function. The Tyr-402 phosphorylated form serves as a docking site for SRC and is important for the organization of the osteoclast actin cytoskeleton and attachment sites and for bone resorption.

Tissue specificity
Most abundant in the brain, with highest levels in amygdala and hippocampus. Low levels in kidney. Also expressed in spleen and lymphocytes.

Sequence similarities
Belongs to the protein kinase superfamily. Tyr protein kinase family. FAK subfamily. Contains 1 FERM domain. Contains 1 protein kinase domain.

Post-translational modifications
Phosphorylated on tyrosine residues in response to various stimuli that elevate the intracellular calcium concentration, as well as by PKC activation. Recruitment by nephrocystin to cell matrix adhesions initiates Tyr-402 phosphorylation. In monocytes, adherence to substrata is required for tyrosine phosphorylation and kinase activation. Angiotensin II, thapsigargin and L-alpha-lysophosphatidic acid (LPA) also induce autophosphorylation and increase kinase activity.
Cellular localization


Images

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: PYK2 knockout HAP1 whole cell lysate (20 µg)
Lane 3: Jurkat whole cell lysate (20 µg)
Lane 4: Hu brain whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32571 observed at 125 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab32571 was shown to specifically recognize PYK2 in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when PYK2 knockout samples were examined. Wild-type and PYK2 knockout samples were subjected to SDS-PAGE. Ab32571 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Immunocytochemistry/Immunofluorescence analysis of PC12 cells labelling PYK2 with unpurified ab32571 at a 1/100 dilution.
**Western blot - Anti-PYK2 antibody [YE353] (ab32571)**

**All lanes**: Anti-PYK2 antibody [YE353] (ab32571) at 1/10000 dilution (purified)

**Lane 1**: Ramos cell lysate  
**Lane 2**: Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**  
**All lanes**: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution  
**Predicted band size**: 116 kDa  
**Observed band size**: 116 kDa

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

**Western blot - Anti-PYK2 antibody [YE353] (ab32571)**

**Anti-PYK2 antibody [YE353] (ab32571) at 10000 cells (purified) + RAW264.7 cell lysate at 20 µg**

**Secondary**  
**Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution**  
**Predicted band size**: 116 kDa  
**Observed band size**: 116 kDa

Blocking buffer and concentration: 5% NFDM/TBST.  
Diluting buffer and concentration: 5% NFDM/TBST.
All lanes: Anti-PYK2 antibody [YE353] (ab32571) at 1/2000 dilution (purified)

Lane 1: Mouse brain tissue lysate
Lane 2: Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 116 kDa
Observed band size: 116 kDa

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

Anti-PYK2 antibody [YE353] (ab32571) at 1/5000 dilution (unpurified) + Jurkat cell lysate

Predicted band size: 116 kDa
Observed band size: 116 kDa
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebral cortex tissue labelling PYK2 with purified ab32571 at 1/300. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebral cortex tissue labelling PYK2 with purified ab32571 at 1/300. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PYK2 antibody [YE353] (ab32571)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebral cortex tissue labelling PYK2 with purified ab32571 at 1/300. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal human brain tissue labelling PYK2 with unpurified ab32571 at a 1/250 dilution.

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling PYK2 with purified ab32571 at 1/60. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. Control: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

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