**Product datasheet**

**Anti-PKC beta 2 antibody [Y125] ab32026**

**Overview**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-PKC beta 2 antibody [Y125]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit monoclonal [Y125] to PKC beta 2</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Specificity</td>
<td>ab32026 recognises PKC beta 2, it does not cross react with human PKC beta 1. Immunogen's homology with PKC alpha is 77.8%. Cross-reactivity with PKC alpha has not been tested.</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: WB, IHC-P, ICC/IF, Flow Cyt</td>
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<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Human, Recombinant fragment</td>
</tr>
<tr>
<td>Predicted to work with</td>
<td>Cow, Pig</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Synthetic peptide within Human PKC beta 2 aa 600-700 (C terminal). The exact sequence is proprietary.</td>
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<tr>
<td>Epitope</td>
<td>ab32026 reacts with an epitope located in the region near the C terminus of PKC beta 2.</td>
</tr>
<tr>
<td>Positive control</td>
<td>K562 cell lysate, HeLa cell lysate, rat spinal cord lysate and human breast carcinoma</td>
</tr>
<tr>
<td>General notes</td>
<td>This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.</td>
</tr>
</tbody>
</table>

**Properties**

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
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<tbody>
<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.</td>
</tr>
</tbody>
</table>
| Storage buffer | pH: 7.20  
Preservative: 0.01% Sodium azide  
Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA |
| Purity | Protein A purified |
Clonality: Monoclonal
Clone number: Y125
Isotype: IgG

Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab32026 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>Abviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★ (4)</td>
<td>1/1000 - 1/10000. Detects a band of approximately 77 kDa (predicted molecular weight: 77 kDa).</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/250 - 1/500.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
</tbody>
</table>

Target

Function
Calcium-activated and phospholipid-dependent serine/threonine-protein kinase involved in various processes such as regulation of the B-cell receptor (BCR) signalosome, apoptosis and transcription regulation. Plays a key role in B-cell activation and function by regulating BCR-induced NF-kappa-B activation and B-cell survival. Required for recruitment and activation of the IKK kinase to lipid rafts and mediates phosphorylation of CARD11/CARMA1 at ‘Ser-559’, ‘Ser-644’ and ‘Ser-652’, leading to activate the NF-kappa-B signaling. Involved in apoptosis following oxidative damage: in case of oxidative conditions, specifically phosphorylates ‘Ser-36’ of isoform p66Shc of SHC1, leading to mitochondrial accumulation of p66Shc, where p66Shc acts as a reactive oxygen species producer. Acts as a coactivator of androgen receptor (ANDR)-dependent transcription, by being recruited to ANDR target genes and specifically mediating phosphorylation of ‘Thr-6’ of histone H3 (H3T6ph), a specific tag for epigenetic transcriptional activation that prevents demethylation of histone H3 ‘Lys-4’ (H3K4me) by LSD1/KDM1A. Also involved in triglyceride homeostasis. Serves as the receptor for phorbol esters, a class of tumor promoters.

Sequence similarities
Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily.
Contains 1 AGC-kinase C-terminal domain.
Contains 1 C2 domain.
Contains 2 phorbol-ester/DAG-type zinc fingers.
Contains 1 protein kinase domain.

Post-translational modifications
Phosphorylation on Thr-500 within the activation loop renders it competent to autophosphorylate. Subsequent autophosphorylation of Thr-642 maintains catalytic competence, and autophosphorylation on Ser-661 appears to release the kinase into the cytosol. Autophosphorylation on other sites i.e. in the N-terminal and hinge regions have no effect on
enzyme activity.

**Cellular localization**

**Images**

Western blot - Anti-PKC beta 2 antibody [Y125] (ab32026)

- Anti-PKC beta 2 antibody [Y125] (ab32026) at 1/500 dilution + Recombinant human PKC beta 2 protein (ab60841) at 0.01 µg

**Secondary**
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

- **Predicted band size**: 77 kDa
- **Observed band size**: 103 kDa

**Exposure time**: 20 seconds

Blocking Buffer: 5% NFDM/TBST
Diluting Buffer: 5% NFDM/TBST

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKC beta 2 antibody [Y125] (ab32026)

- ab32026, at a 1/100 dilution, staining human PKC beta 2 in breast carcinoma by Immunohistochemistry, Paraffin embedded tissue
- Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Immunocytochemistry/ Immunofluorescence - Anti-PKC beta 2 antibody [Y125] (ab32026)

- ab32026, at a 1/250 dilution, staining human PKC beta 2 in HeLa cells by Immunofluorescence
Overlay histogram showing HeLa cells stained with ab32026 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32026, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

**All lanes**: Anti-PKC beta 2 antibody [Y125] (ab32026) at 1/500 dilution

**Lane 1**: K562 whole cell lysates at 20 µg
**Lane 2**: Active human PKC beta 2 full length protein at 0.02 µg
**Lane 3**: Human fetal brain at 20 µg
**Lane 4**: NIH/3T3 whole cell lysates at 20 µg
**Lane 5**: Raw264.7 whole cell lysates at 20 µg

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size**: 77 kDa
**Observed band size**: 77 kDa

**Exposure time**: 15 seconds

Blocking buffer: 5% NFDM/TBST
Diluting buffer: 5% NFDM/TBST

The PKC beta 2 recombinant protein is GST-tagged, so the molecular weight is higher.
Antibody used: Anti-PKC beta 2 antibody [Y125] (ab32026)

**Dilution and Sample**
- 1/500 dilution of antibody + Rat spinal cord at 20 µg

**Secondary**
- Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 77 kDa
**Observed band size:** 77 kDa
**Exposure time:** 3 minutes

**Blocking and Dilution Buffers**
- Blocking Buffer: 5% NFDM/TBST
- Diluting Buffer: 5% NFDM/TBST

**Additional Blot**
- Anti-PKC beta 2 antibody [Y125] (ab32026) at 1/500 dilution + K562 cell lysate

**Predicted band size:** 77 kDa
**Observed band size:** 77 kDa
All lanes: Anti-PKC beta 2 antibody [Y125] (ab32026) at 1/1000 dilution

Lane 1: MCF-7 whole cell lysates
Lane 2: HeLa whole cell lysates
Lane 3: K562 whole cell lysates
Lane 4: Recombinant human PKC alpha protein (Active) (ab55672)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 77 kDa
Observed band size: 77 kDa

Exposure time: 30 seconds

Blocking Buffer: 5% NFDM/TBST
Diluting Buffer: 5% NFDM/TBST
Anti-PKC beta 2 antibody [Y125] (ab32026)

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