**Product datasheet**

**Anti-NCX1 antibody [C2C12] ab2869**

**Overview**

- **Product name**: Anti-NCX1 antibody [C2C12]
- **Description**: Mouse monoclonal [C2C12] to NCX1
- **Host species**: Mouse
- **Tested applications**: Suitable for: IHC-P, ICC/IF, IHC-Fr, ELISA, IP, WB, Flow Cyt
- **Species reactivity**: Reacts with: Mouse, Rat, Rabbit, Guinea pig, Dog, Human, Pig
- **Immunogen**: Full length native protein (purified) corresponding to Dog NCX1. Purified from canine cardiac sodium/calcium exchanger.
- **Epitope**: This antibody recognizes an epitope between amino acids 371-525 on the intracellular side of the plasma membrane.

**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 or -80°C. Avoid freeze / thaw cycle.
- **Storage buffer**: Preservative: 0.05% Sodium azide
  Constituent: PBS
- **Purity**: Immunogen affinity purified
- **Primary antibody notes**: The sodium/calcium exchanger of cardiac sarcolemma rapidly transports calcium during excitation-contraction coupling and is the dominant myocardial calcium efflux mechanism. The sodium/calcium exchanger uses the transmembrane sodium gradient to catalyze countertransport of calcium against its electrochemical gradient in a 3 sodium : 1 calcium exchange. Sodium/calcium exchange activity is present in excitable cells and in non-excitable cells.
- **Clonality**: Monoclonal
- **Clone number**: C2C12
- **Isotype**: IgM

**Applications**

Our Abpromise guarantee covers the use of ab2869 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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<tbody>
<tr>
<td>IHC-P</td>
<td>1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
<td></td>
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<tr>
<td>ICC/IF</td>
<td>1/200.</td>
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<tr>
<td>IHC-Fr</td>
<td>Use at an assay dependent concentration. PubMed: 21408028</td>
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<tr>
<td>ELISA</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IP</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>1/1000. By Western blot, this antibody detects a 120 kDa protein representing the sodium/calcium exchanger from guinea pig cardiac extract. The bands seen at 70 kDa and 160 kDa represent a proteolytic fragment and non-reduced exchanger respectively. This antibody is not recommended for Western blot procedures of rat tissues.</td>
<td></td>
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</table>
| Flow Cyt    | 1/20 - 1/100.  
**ab91545** - Mouse monoclonal IgM, is suitable for use as an isotype control with this antibody. |

**Target**

**Function**
Rapidly transports Ca(2+) during excitation-contraction coupling. Ca(2+) is extruded from the cell during relaxation so as to prevent overloading of intracellular stores.

**Tissue specificity**
Expressed in cardiac sarcolemma, brain, kidney, liver, pancreas, skeletal muscle, placenta and lung.

**Sequence similarities**
Belongs to the sodium/potassium/calcium exchanger family. SLC8 subfamily. Contains 2 CaX-beta domains.

**Cellular localization**
Cell membrane.
ab2869 staining NCX1 in Human kidney tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde, permeabilized with 0.05% Tween20 and blocked with 5% normal goat serum in 1XPBS + 0.05% Tween20 for 1 hour at 25°C; antigen retrieval was by heat mediation in sodium citrate (pH 6.0) buffer. Samples were incubated with primary antibody (1/100 in blocking buffer) for 1 hour at 25°C. Ab47827 (1/500) was used as the secondary antibody.

Overlay histogram showing HEK293 cells stained with ab2869 (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 (ab2869, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgM (mu chain) (ab97007) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgM [ICIGM] (ab91545, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.
Immunocytochemistry/Immunofluorescence analysis of NCX1 shows staining in A2058 cells. NCX1 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab2869 (1:100) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.

Immunocytochemistry/Immunofluorescence analysis of NCX1 shows staining in A549 cells. NCX1 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab2869 (1:100) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.

Immunocytochemistry/Immunofluorescence analysis of NCX1 shows staining in U251 cells. NCX1 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab2869 (1:100) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.
This image shows Human embryonic stem cell derived cardiomyocytes, stained with dapi (blue) and anti-NCX1 antibody ab2869 (red). The cells were fixed in paraformaldehyde, permeabilized with 0.1% Triton X-100 in PBS and blocked with 4% goat serum for 1 hour. The cells were then incubated with primary antibody (1/100) for 16 hours at 4°C.

Immunohistochemistry was performed on normal deparaffinized Human kidney tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:50 with a mouse monoclonal antibody recognizing Sodium/Calcium Exchanger ab2869 or without primary antibody (negative control; right panel) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.
Immunohistochemistry was performed on normal deparaffinized Human heart tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a mouse monoclonal antibody recognizing Sodium/Calcium Exchanger ab2869 or without primary antibody (negative control; right panel) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

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