## Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-GM130 antibody [EP892Y] - cis-Golgi Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit monoclonal [EP892Y] to GM130 - cis-Golgi Marker</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Specificity</td>
<td>Mouse and rat cell lines pc12, 3t3, raw 264.7 were tested positive in WB. However, brain, kidney, spleen and heart were negative from the two species.</td>
</tr>
</tbody>
</table>

**Tested applications**

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

**Species reactivity**

**Reacts with:** Cow, Dog, Human, Monkey, African green monkey

**Does not react with:** Mouse, Rat

**Immunogen**

Synthetic peptide within Human GM130 aa 1-100. The exact sequence is proprietary.

**Positive control**

WB: HeLa, MCF7, MDCK(NBL-2), MDBK(BL-1) and COS-1 cell lysates. IHC-P: Human cervix carcinoma and liver tissues. ICC/IF: HeLa cells, ARPE-19 cells, Bovine brain microvascular endothelial cells, and monkey kidney cells. Flow Cyt: HeLa 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

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.</td>
</tr>
</tbody>
</table>
| Storage buffer | pH: 7.20  
Preservative: 0.01% Sodium azide  
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA |
Purity: Protein A purified
Clonality: Monoclonal
Clone number: EP892Y
Isotype: IgG

Function: Golgi auto-antigen; probably involved in maintaining cis-Golgi structure.
Sequence similarities: Belongs to the GOLGA2 family.
Domain: Extended rod-like protein with coiled-coil domains.
Cellular localization: Golgi apparatus > Golgi stack membrane.

Applications

Our Abpromise guarantee covers the use of ab52649 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>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/50 - 1/250. PFA fixation should be most suitable.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐🟥</td>
<td>1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. Overnight incubation is recommended.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐🟥</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/20.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐🟥</td>
<td>1/1000 - 1/10000. Detects a band of approximately 140 kDa (predicted molecular weight: 112 kDa).</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐🟥</td>
<td>1/20 - 1/50.</td>
</tr>
</tbody>
</table>

Target

Function: Golgi auto-antigen; probably involved in maintaining cis-Golgi structure.
Sequence similarities: Belongs to the GOLGA2 family.
Domain: Extended rod-like protein with coiled-coil domains.
Cellular localization: Golgi apparatus > Golgi stack membrane.

Images
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling GM130 with purified ab52649 at 1/50. 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. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/50) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

**All lanes** : Anti-GM130 antibody [EP892Y] - cis-Golgi Marker (ab52649) at 1/5000 dilution (purified)

**Lane 1** : MDCK(NBL-2) cell lysate

**Lane 2** : MDCK(BL-1) cell lysate

**Lane 3** : COS-1 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**: 112 kDa

**Observed band size**: 130 kDa

why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling GM130 with purified ab52649 at 1/100. 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.

ab52649 (purified) at 1/20 immunoprecipitating GM130 in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg).

Lane 2 (+): ab52649 + HeLa whole cell lysate (10µg).

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab52649 in HeLa whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP (HRP) was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.
Flow cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling GM130 (red) with ab52649 at a 1/20 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (ab172730). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.

**Effect of Irgm1 palmitoylation mutation on Golgi association**

Irgm1 KO MEF were transfected with plasmids expressing wild-type or mutant Irgm1 proteins, as indicated. The cells were exposed to 100 U/ml IFN-γ for 24 h, stained with anti-Irgm1 and anti-GM130 antibodies, and used for immunofluorescence analysis. The experiment was performed 3 times, with at least 20 cells analyzed per group in each experiment. (A) Shown are images from representative cells. The scale bar represents 20 µm.

Cells are 4% paraformaldehyde fixed, 0.2% saponin-permeabilized.

**All lanes** : Anti-GM130 antibody [EP892Y] - cis-Golgi Marker (ab52649) at 1/1000 dilution (purified)

**Lane 1** : HeLa cell lysate

**Lane 2** : MCF7 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**: 112 kDa
Observed band size: 130 kDa. Why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling GM130 with unpurified ab52649 at a dilution of 1/500.

Unpurified ab52649 staining GM130 in Bovine brain microvascular endothelial cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% saponin and blocked with 5% BSA for 90 minutes at 37°C. Samples were incubated with primary antibody (1/100 in 0.1% saponin + 1% BSA) for 18 hours at 4°C. An undiluted Alexa Fluor® 568-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.
Anti-GM130 antibody [EP892Y] - cis-Golgi Marker (ab52649) at 1/200000 dilution (unpurified) + HeLa cell lysate at 10 µg

**Secondary**
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

**Predicted band size:** 112 kDa

Unpurified ab52649 staining GM130 (magenta) in monkey kidney cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and blocked with 3% BSA + 0.5% Triton X-100 for 45 minutes at 25°C. Samples were incubated with primary antibody (1/1500 in 3% BSA + 0.5% Triton X-100) for 45 minutes at 25°C. An Alexa Fluor® 647-conjugated donkey anti-rabbit IgG polyclonal (2 µg/ml) was used as the secondary antibody. Nuclei stained with Picogreen.
(ab52649)

This image is courtesy of an Abreview submitted by Dr Vladimir Milenkovic

Unpurified ab52649 staining GM130 in human ARPE-19 cells by ICC/IF (immunocytochemistry/immunofluorescence). Cells were formaldehyde fixed, permeabilized by 0.5% TX-100 and blocked with 5% serum for 20 minutes at 25°C. The sample was incubated with the primary antibody (1/500 in 1% goat serum, 0.1%TX100, 1 x PBS) for 16 hours at 4°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit polyclonal (1/500) was used as the secondary.

ICC/IF image of unpurified ab52946 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab52946, 1µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-rabbit IgG - H&L, pre-adsorbed (ab96899) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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