Product datasheet

Anti-Glutathione Synthetase antibody [EPR6563]  
ab133592

Overview

**Product name**  Anti-Glutathione Synthetase antibody [EPR6563]

**Description**  Rabbit monoclonal [EPR6563] to Glutathione Synthetase

**Host species**  Rabbit

**Tested applications**  Suitable for: WB, IHC-P, Flow Cyt  
Unsuitable for: IP

**Species reactivity**  Reacts with: Human  
Predicted to work with: Mouse, Rat

**Immunogen**  Synthetic peptide within Human Glutathione Synthetase aa 350-450 (internal sequence). The exact sequence is proprietary.  
(Peptide available as ab211544)

**Positive control**  293T, HeLa, Daudi and HT-1080 cell lysates; Human colon tissue

**General notes**  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.

Properties

**Form**  Liquid

**Storage instructions**  Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

**Dissociation constant (K_D)**  \( K_D = 2.74 \times 10^{-11} \) M
Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EPR6563

Isotype
IgG

Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab133592 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>WB</td>
<td></td>
<td>1/1000 - 1/10000. Predicted molecular weight: 52 kDa.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/100 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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</table>

Application notes
Is unsuitable for IP.

Target

Pathway
Sulfur metabolism; glutathione biosynthesis; glutathione from L-cysteine and L-glutamate: step 2/2.

Involvement in disease
Defects in GSS are the cause of glutathione synthetase deficiency (GSS deficiency) [MIM:266130]; also known as 5-oxoprolinuria or pyroglutamic aciduria. It is a severe form characterized by an increased rate of hemolysis and defective function of the central nervous system.
Defects in GSS are the cause of glutathione synthetase deficiency of erythrocytes (GLUSYNDE) [MIM:231900]. Glutathione synthetase deficiency of erythrocytes is a mild form causing hemolytic anemia.

Sequence similarities
Belongs to the eukaryotic GSH synthase family.
Western blot - Anti-Glutathione Synthetase antibody [EPR6563] (ab133592)

**All lanes**: Anti-Glutathione Synthetase antibody [EPR6563] (ab133592) at 1/1000 dilution

**Lane 1**: Wild-type HEK-293T cell lysate

**Lane 2**: GSS knockout HEK-293T cell lysate

**Lane 3**: HeLa cell lysate

**Lane 4**: Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size**: 52 kDa

**Observed band size**: 50 kDa

**Lanes 1-4**: Merged signal (red and green). Green - ab133592 observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab133592 was shown to react with GSS in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266342 (knockout cell lysate ab257460) was used. Wild-type HEK-293T and GSS knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk.

ab133592 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.
Overlay histogram showing Jurkat cells stained with ab133592 (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 (ab133592, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1μg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Immunohistochemical analysis of paraffin-embedded Human colon tissue labelling Glutathione Synthetase with ab133592 at 1/50 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D
Western blot - Anti-Glutathione Synthetase antibody [EPR6563] (ab133592)

**All lanes**: Anti-Glutathione Synthetase antibody [EPR6563] (ab133592) at 1/1000 dilution

- **Lane 1**: 293T cell lysate
- **Lane 2**: HeLa cell lysate
- **Lane 3**: HT-1080 cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

- **All lanes**: HRP labelled Goat anti-Rabbit IgG at 1/2000 dilution

**Predicted band size**: 52 kDa

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