abcam

Product datasheet

Anti-Glutathione Synthetase antibody [EPR6563] ab133592

KO VALIDATED Recombinant RabMAb

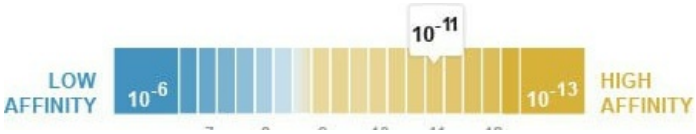
7 References 6 Images

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 (Intra) **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



Learn more about K_D

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.

Application	Abreviews	Notes
WB		1/1000 - 1/10000. Predicted molecular weight: 52 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/100 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Application notes Is unsuitable for IP.

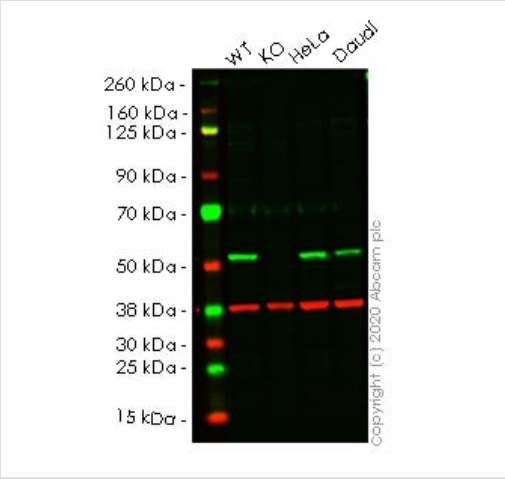
Target

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.

Images



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

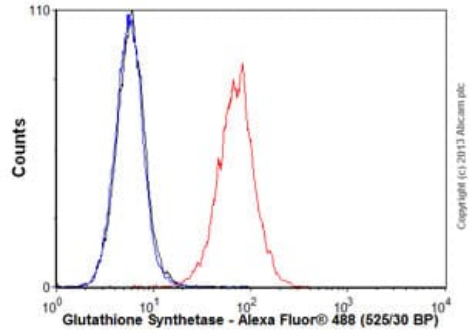
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 50 kDa

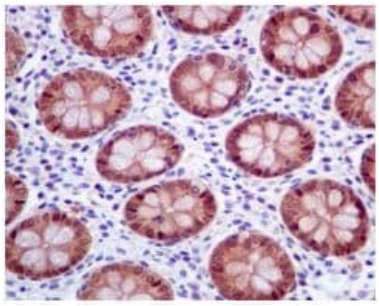
Lanes 1- 4: Merged signal (red and green). Green - ab133592 observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

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.



Flow Cytometry (Intracellular) - Anti-Glutathione Synthetase antibody [EPR6563] (ab133592)

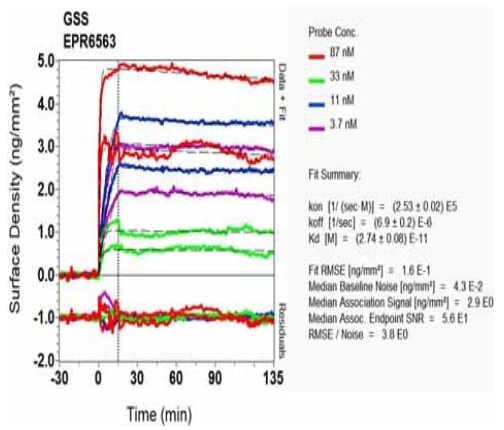
Overlay histogram showing Jurkat cells stained with ab133592 (red line). The cells were fixed with 80% methanol (5 min)/ and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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/1x10^6 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glutathione Synthetase antibody [EPR6563] (ab133592)

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.

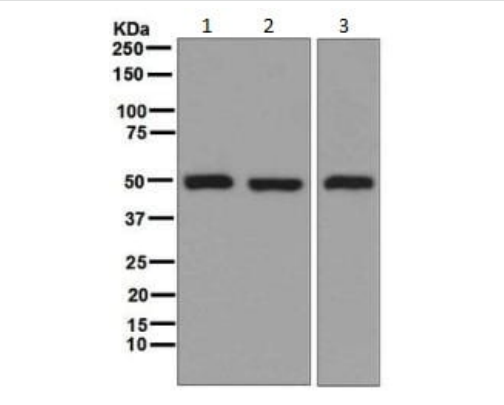


OI-RD Scanning - Anti-Glutathione Synthetase antibody [EPR6563] (ab133592)

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)

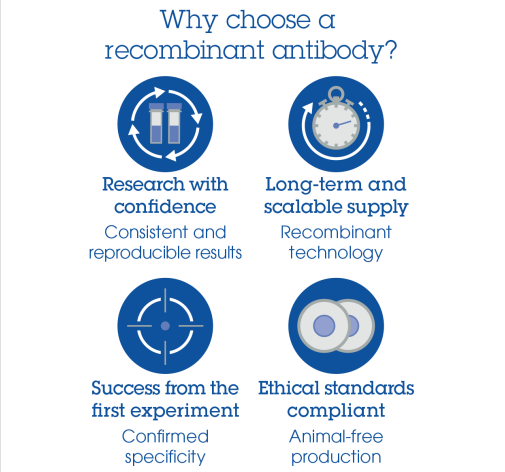
All lanes : Anti-Glutathione Synthetase antibody [EPR6563] (ab133592) at 1/1000 dilution

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



Anti-Glutathione Synthetase antibody [EPR6563]

(ab133592)

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