abcam

Product datasheet

Anti-MGEA5/OGA antibody [EPR7154(B)] - BSA and Azide free ab227989





1 References 6 Images

Overview

Product name Anti-MGEA5/OGA antibody [EPR7154(B)] - BSA and Azide free

Rabbit monoclonal [EPR7154(B)] to MGEA5/OGA - BSA and Azide free **Description**

Host species Rabbit

Tested applications Suitable for: IP, IHC-P, WB

Unsuitable for: Flow Cyt or ICC/IF

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab155521)

Positive control IHC-P: Human kidney and colon tissues. IP: HeLa cells. WB: HeLa, JAR, and HEK-293T cell

General notes ab227989 is the carrier-free version of ab124807.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

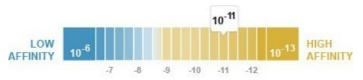
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Dissociation constant (K_D) $K_D = 9.80 \times 10^{-11} M$



Learn more about K_D

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR7154(B)

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab227989 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
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 130 kDa (predicted molecular weight: 102 kDa).

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Isoform 1: Cleaves GlcNAc but not GalNAc from O-glycosylated proteins. Can use p-nitrophenyl-

beta-GlcNAc and 4-methylumbelliferone-GlcNAc as substrates but not p-nitrophenyl-beta-GalNAc or p-nitrophenyl-alpha-GlcNAc (in vitro) (PubMed:11148210). Does not bind acetyl-CoA and does

not have histone acetyltransferase activity (PubMed:24088714).

lsoform 3: Cleaves GlcNAc but not GalNAc from O-glycosylated proteins. Can use p-nitrophenyl-beta-GlcNAc as substrate but not p-nitrophenyl-beta-GalNAc or p-nitrophenyl-alpha-GlcNAc (in

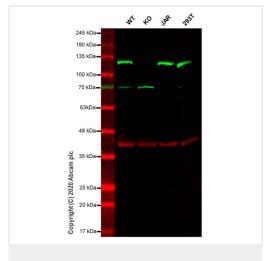
vitro), but has about six times lower specific activity than isoform 1.

Tissue specificity Ubiquitous. Shows highest expression in the brain, placenta and pancreas.

Sequence similaritiesBelongs to the glycosyl hydrolase 84 family.

Post-translational Proteolytically cleaved by caspase-3 during apoptosis. The fragments interact with each other;

Images



Western blot - Anti-MGEA5/OGA antibody [EPR7154(B)] - BSA and Azide free (ab227989) **All lanes :** Anti-MGEA5/OGA antibody [EPR7154(B)] (**ab124807**) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: MGEA5 knockout HeLa cell lysate

Lane 3: JAR cell lysate

Lane 4: HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

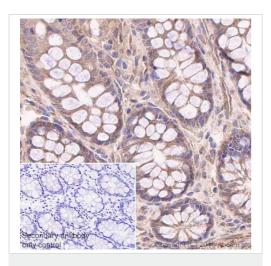
Performed under reducing conditions.

Predicted band size: 102 kDa Observed band size: 130 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab124807</u>).

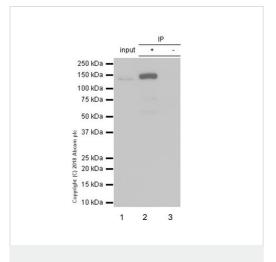
Lanes 1-4: Merged signal (red and green). Green - <u>ab124807</u> observed at 130 kDa. Red - loading control, <u>ab8245</u> observed at 37 kDa.

ab124807 Anti-MGEA5/OGA antibody [EPR7154(B)] was shown to specifically react with MGEA5/OGA in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265821 (knockout cell lysate ab257263) was used. Wild-type and MGEA5/OGA knockout samples were subjected to SDS-PAGE. ab124807 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MGEA5/OGA antibody
[EPR7154(B)] - BSA and Azide free (ab227989)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon tissue sections labeling MGEA5/OGA with purified ab124807 at 1:50 dilution (12.56 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control:PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunoprecipitation - Anti-MGEA5/OGA antibody [EPR7154(B)] - BSA and Azide free (ab227989)

<u>ab124807</u> (purified) at 1:30 dilution (2μg) immunoprecipitating MGEA5/OGA in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

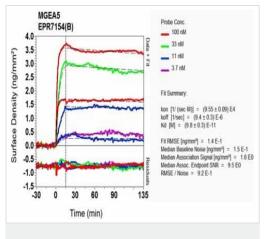
Lane 2 (+): ab124807 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of

ab124807 in HeLa whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



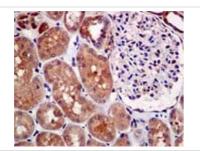
Ol-RD Scanning - Anti-MGEA5/OGA antibody [EPR7154(B)] - BSA and Azide free (ab227989)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab124807</u>).

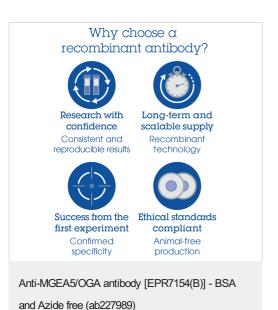


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MGEA5/OGA antibody
[EPR7154(B)] - BSA and Azide free (ab227989)

This IHC data was generated using the same anti-MGEA5/OGA antibody clone, EPR7154(B), in a different buffer formulation (cat# <u>ab124807</u>).

ab124807, at 1/100 dilution, staining MGEA5 in paraffin-embedded Human kidney tissue by Immunohistochemistry.

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



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