abcam

Product datasheet

Anti-GBA antibody [EPR5142] ab125065

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-GBA antibody [EPR5142]
Description	Rabbit monoclonal [EPR5142] to GBA
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, IHC-Fr **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human GBA aa 50-150. The exact sequence is proprietary.
Positive control	IHC-P: Human lung cancer and colon tissue; WB: Saos-2, HAP1, HepG2, MCF7, U-87 MG, HeLa and SH-SY5Y cell lysates. IHC-Fr: Frozen human hippocampus and placenta tissue sections.
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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5142

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab125065 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. Detects a band of approximately 60 kDa (predicted molecular weight: 60 kDa).
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IHC-Fr		Use a concentration of 5 µg/ml.

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

Target

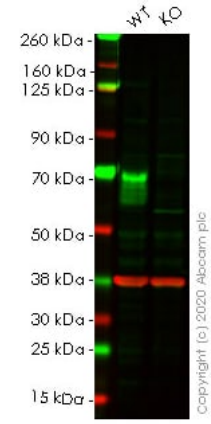
Involvement in disease Defects in GBA are the cause of Gaucher disease (GD) [MIM:230800]; also known as glucocerebrosidase deficiency. GD is the most prevalent lysosomal storage disease, characterized by accumulation of glucosylceramide in the reticulo-endothelial system. Different clinical forms are recognized depending on the presence (neuronopathic forms) or absence of central nervous system involvement, severity and age of onset.
Defects in GBA are the cause of Gaucher disease type 1 (GD1) [MIM:230800]; also known as adult non-neuronopathic Gaucher disease. GD1 is characterized by hepatosplenomegaly with consequent anemia and thrombopenia, and bone involvement. The central nervous system is not involved.
Defects in GBA are the cause of Gaucher disease type 2 (GD2) [MIM:230900]; also known as acute neuronopathic Gaucher disease. GD2 is the most severe form and is universally progressive and fatal. It manifests soon after birth, with death generally occurring before patients reach two years of age.
Defects in GBA are the cause of Gaucher disease type 3 (GD3) [MIM:231000]; also known as subacute neuronopathic Gaucher disease. GD3 has central nervous manifestations.
Defects in GBA are the cause of Gaucher disease type 3C (GD3C) [MIM:231005]; also known as pseudo-Gaucher disease or Gaucher-like disease.
Defects in GBA are the cause of Gaucher disease perinatal lethal (GDPL) [MIM:608013]. It is a distinct form of Gaucher disease type 2, characterized by fetal onset. Hydrops fetalis, in utero fetal death and neonatal distress are prominent features. When hydrops is absent, neurologic involvement begins in the first week and leads to death within 3 months. Hepatosplenomegaly is a major sign, and is associated with ichthyosis, arthrogryposis, and facial dysmorphism.
Note=Perinatal lethal Gaucher disease is associated with non-immune hydrops fetalis, a generalized edema of the fetus with fluid accumulation in the body cavities due to non-immune causes. Non-immune hydrops fetalis is not a diagnosis in itself but a symptom, a feature of many genetic disorders, and the end-stage of a wide variety of disorders.
Defects in GBA contribute to susceptibility to Parkinson disease (PARK) [MIM:168600]. A complex neurodegenerative disorder characterized by bradykinesia, resting tremor, muscular

rigidity and postural instability. Additional features are characteristic postural abnormalities, dysautonomia, dystonic cramps, and dementia. The pathology of Parkinson disease involves the loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies (intraneuronal accumulations of aggregated proteins), in surviving neurons in various areas of the brain. The disease is progressive and usually manifests after the age of 50 years, although early-onset cases (before 50 years) are known. The majority of the cases are sporadic suggesting a multifactorial etiology based on environmental and genetic factors. However, some patients present with a positive family history for the disease. Familial forms of the disease usually begin at earlier ages and are associated with atypical clinical features.

Sequence similarities Belongs to the glycosyl hydrolase 30 family.

Cellular localization Lysosome membrane. Interaction with saposin-C promotes membrane association.

Images



Western blot - Anti-GBA antibody [EPR5142] (ab125065)

All lanes : Anti-GBA antibody [EPR5142] (ab125065) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : GBA knockout HeLa cell lysate

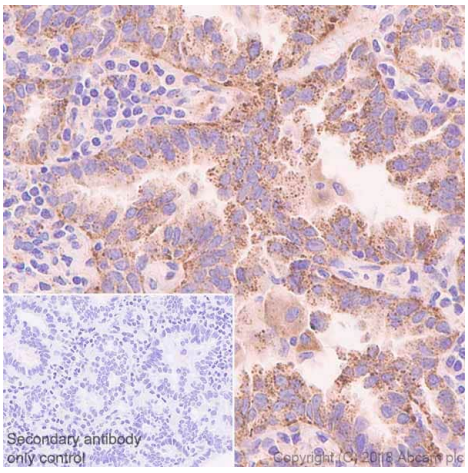
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 70 kDa

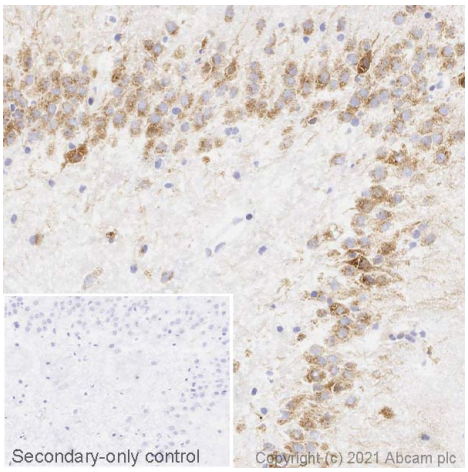
Lanes 1- 2: Merged signal (red and green). Green - ab125065 observed at 70 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab125065 was shown to react with GBA in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265038** (knockout cell lysate **ab256929**) was used. Wild-type HeLa and GBA knockout HeLa 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. ab125065 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GBA antibody [EPR5142] (ab125065)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung cancer tissue sections labeling GBA with Purified ab125065 at 1:50 dilution (2.1 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.

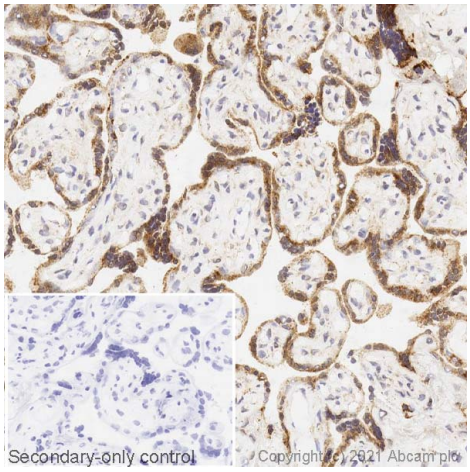


Immunohistochemistry (Frozen sections) - Anti-GBA antibody [EPR5142] (ab125065)

IHC image of GBA staining in a section of frozen normal human hippocampus* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab125065, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

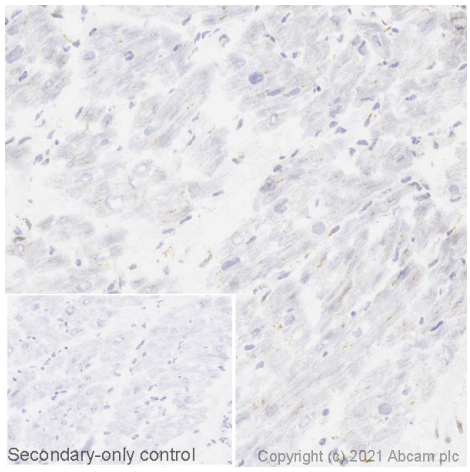


Immunohistochemistry (Frozen sections) - Anti-GBA antibody [EPR5142] (ab125065)

IHC image of GBA staining in a section of frozen normal human placenta* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab125065, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

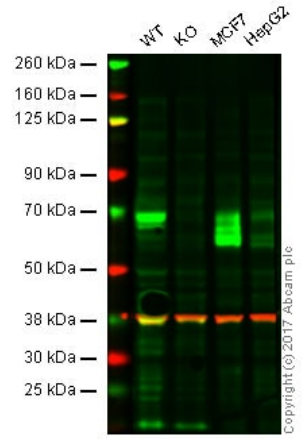


Immunohistochemistry (Frozen sections) - Anti-GBA antibody [EPR5142] (ab125065)

Negative control image: IHC image of GBA staining in a section of frozen normal human heart* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab125065, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Western blot - Anti-GBA antibody [EPR5142] (ab125065)

All lanes : Anti-GBA antibody [EPR5142] (ab125065) at 1/1000 dilution

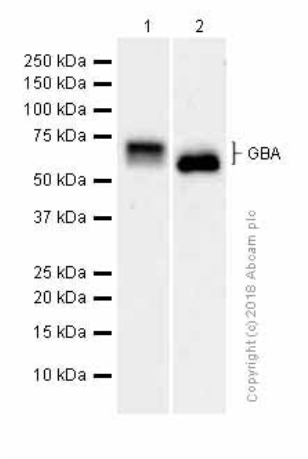
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : GBA knockout HAP1 whole cell lysate
Lane 3 : MCF7 whole cell lysate
Lane 4 : HepG2 whole cell lysate

Lysates/proteins at 40 µg per lane.

Predicted band size: 60 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab125065 observed at 70 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab125065 was shown to specifically recognize GBA in wild-type HAP1 cells as well as additional cross-reactive bands. No bands were observed when GBA knockout samples were used. Wild-type and GBA knockout samples were subjected to SDS-PAGE. Ab125065 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-GBA antibody [EPR5142] (ab125065)

All lanes : Anti-GBA antibody [EPR5142] (ab125065) at 1/1000 dilution (Purified)

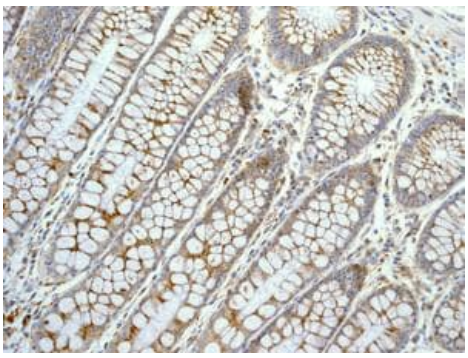
Lane 1 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysates
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 60 kDa
Observed band size: 60-70 kDa

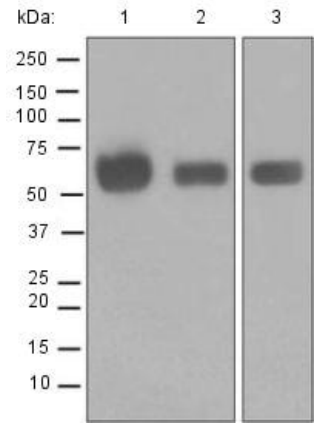
The multiple bands are consistent with what have been described in the literature PMID: 2495719 due to the glycosylation



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GBA antibody [EPR5142] (ab125065)

ab125065, at a 1/50 dilution, staining GBA in paraffin-embedded Human colon tissue by immunohistochemistry. Heat mediated antigen retrieval was permorded using citrate buffer pH 6 before commencing with IHC staining protocol.

This image was generated using the unpurified version of the product.



Western blot - Anti-GBA antibody [EPR5142] (ab125065)

All lanes : Anti-GBA antibody [EPR5142] (ab125065) at 1/1000 dilution

Lane 1 : Saos-2 cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : SH-SY5Y cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

Developed using the ECL technique.

Predicted band size: 60 kDa

This image was generated using the unpurified version of the product.

Anti-GBA antibody [EPR5142] (ab125065)

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