abcam

Product datasheet

Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] ab202665

Recombinant RabMAb

5 Images

Overview

Product name	Anti-O-Linked N-Acetylglucosamine antibody [EPR19847]
Description	Rabbit monoclonal [EPR19847] to O-Linked N-Acetylglucosamine
Host species	Rabbit
Tested applications	**Suitable for:** WB, Dot blot, IP
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide within Human O-Linked N-Acetylglucosamine. The exact sequence is proprietary.
Positive control	WB: HeLa treated with DMSO (0.4%) as baseline control whole cell lysate; HeLa treated with 200 µM Ac45SGlcNAc for 24 hours whole cell lysate; HeLa treated with 200 µM Thiamet-G for 24 hours whole cell lysate; HEK-293t and HeLa whole cell lysate. Rat brain lysate. PC-12 (rat adrenal gland pheochromocytoma). SH-SY5Y (human neuroblastoma epithelial cell). IP: HeLa whole cell lysate.
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. This product is a recombinant rabbit monoclonal antibody.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19847
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab202665** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

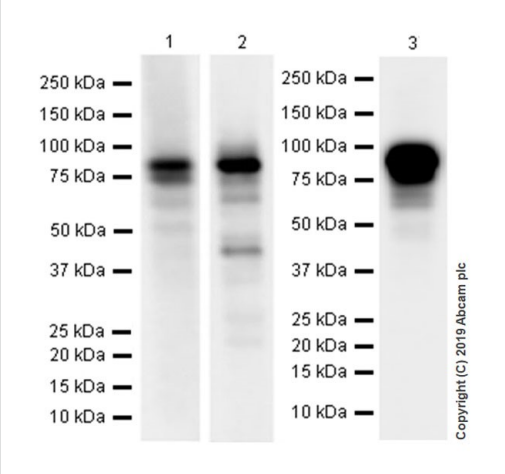
Application	Abreviews	Notes
WB		1/1000.
Dot blot		1/1000.
IP		1/30.

Target

Relevance

Many cellular proteins, including nuclear pore, oncogene, cytoskeletal, heat shock, viral and transcription regulatory proteins contain single O-linked N-acetylglucosamine (O-GlcNAc) residues attached to serine or threonine residues. It has been observed that O-GlcNAc glycosylated proteins tend to be under phosphorylated relative to unglycosylated proteins and that O-GlcNAc bearing proteins tend to be found in multimeric complexes. This has led to the suggestion that O-GlcNAc glycosylation may obscure phosphorylation sites and acts as a signaling mechanism or mediator of signaling.

Images



Western blot - Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665)

All lanes : Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665) at 1/1000 dilution

Lane 1 : Rat brain lysate
Lane 2 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 3 : SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate

Lysates/proteins at 10 µg per lane.

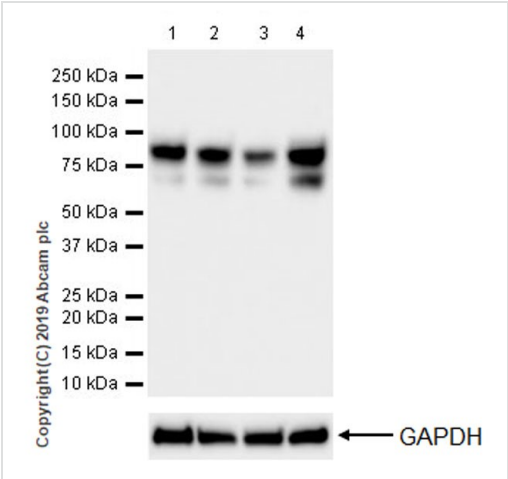
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Observed band size: 20-100 kDa
why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST
Diluting buffer and concentration: 5% NFDM/TBST

Exposure time:

Lane 1:8 seconds;
Lane 2:3 seconds;
Lane 3:26 seconds.



Western blot - Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665)

All lanes : Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665) at 1/1000 dilution

Lane 1 : Untreated HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell) treated with DMSO (0.4%) as baseline control. whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) treated with 200 µM Ac45SGlcNAc for 24 hours whole cell lysate
Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell) treated with 200 µM Thiamet-G for 24 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Observed band size: 50-100 kDa why is the actual band size different from the predicted?

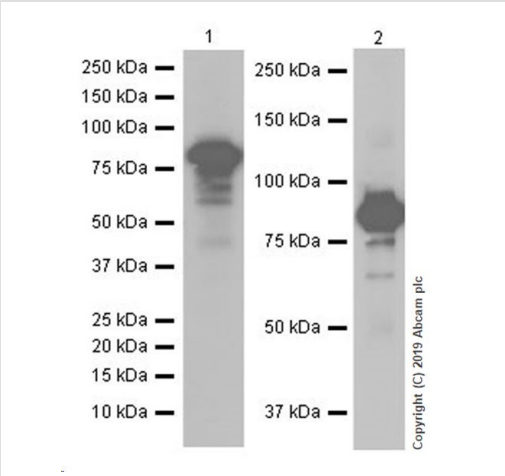
Exposure time: 3 seconds

Blocking buffer and concentration 5% NFDM/TBST
Diluting buffer and concentration 5% NFDM/TBST

Ac45SGlcNAc, an inhibitor of OGT, decreases O-GlcNAc modification. Thiamet-G, an inhibitor of OGA, increases O-GlcNAc modification.

These two chemicals were kindly provided by our collaborator Dr. Xing Chen, Peking University.

The expression pattern is consistent with reference (PMID: 27716624)



Western blot - Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665)

All lanes : Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665) at 1/1000 dilution

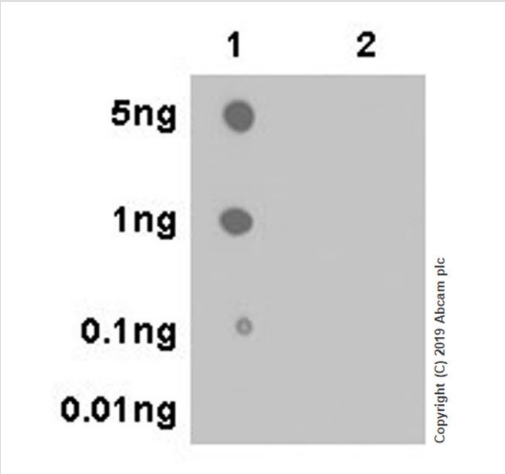
Lane 1 : HEK-293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1: 4 seconds; Lane 2: 15 seconds.



Dot Blot - Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665)

Dot Blot - Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665 1:1000 dilution).

Lane 1: O-linked N-acetylglucosamine (O-GlcNAc) peptide 10 ng.

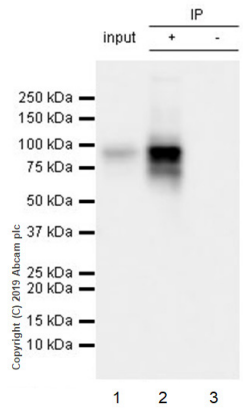
Lane 2: Non-O-GlcNAc peptide 10 ng.

Blocking/Dilution buffer: 5% NFDM/TBST.

Secondary: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051 1:100,000 dilution).

Exposure time of 3 minutes.

The peptides were kindly provided by our collaborator Dr. Xing Chen, Peking University.



Immunoprecipitation - Anti-O-Linked N-Acetylglucosamine antibody [EPR19847] (ab202665)

O-Linked N-Acetylglucosamine was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with ab202665 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab202665 at 1/1,000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.
Lane 1: HeLa whole cell lysate 10 µg (Input).
Lane 2: ab202665 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab202665 in HeLa whole cell lysate.
Blocking/Dilution buffer: 5% NFDM/TBST.
Exposure time: 3 seconds.