abcam

Product datasheet

Anti-O-Linked N-Acetylglucosamine antibody [RL2] ab2739

★★★★★ 10 Abreviews 69 References 4 Images

Overview

Product name	Anti-O-Linked N-Acetylglucosamine antibody [RL2]
Description	Mouse monoclonal [RL2] to O-Linked N-Acetylglucosamine
Host species	Mouse
Specificity	The antibody recognizes an epitope containing (serine/threonine) O-Linked N-Acetylglucosamine, which is found on hundreds of nuclear and cytoplasmic proteins, including “FG” nucleoporins of the nuclear pore complex. The sugar is a key part of the epitope. The antibody detects nuclear pore complex (NPC), cytoplasmic and intranuclear O-Linked glycoproteins from human, mouse, and rat tissues. In Western blot, many bands are expected as the O-Linked N-Acetylglucosamine modification can occur on proteins of different sizes.
Tested applications	**Suitable for:** ICC/IF, IHC-Fr, ChIP/Chip, Dot blot, WB, IP
Immunogen	Tissue, cells or virus corresponding to O-Linked N-Acetylglucosamine. Specifically, isolated rat liver nuclear envelopes, which contain 8 O-Linked glycoproteins in the nuclear pore complex
Positive control	ICC-IF: MCF7 cells. WB: Jurkat cells treated with 50 uM PugNAc; SH-SY5Y) whole cell lysate - treated with 50µM z-Pugnac; Rat Liver Nuclear Envelope lysate.
General notes	This antibody clone [RL2] is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	IgG fraction
Clonality	Monoclonal
Clone number	RL2

Isotype	IgG1
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab2739** in the following tested applications.

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

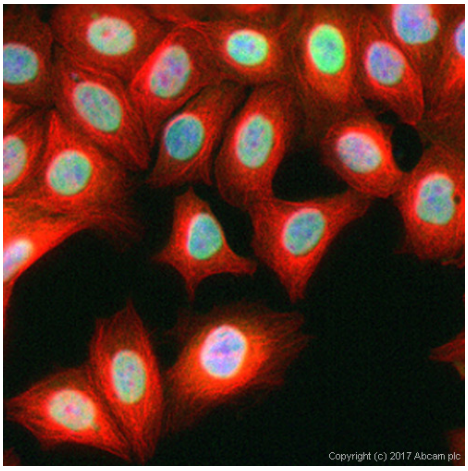
Application	Abreviews	Notes
ICC/IF	★★★★★	Use a concentration of 5 - 10 µg/ml.
IHC-Fr		Use at an assay dependent concentration. PubMed: 23734074
ChIP/Chip		Use at an assay dependent concentration. PubMed: 20368426
Dot blot		1/800.
WB	★★★★★	Use a concentration of 1 µg/ml.
IP		Use at an assay dependent concentration.

Target

Relevance

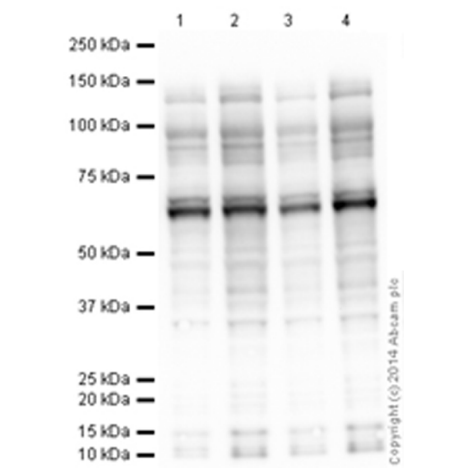
Many cellular proteins, including nuclear pore, oncogene, cytoskeletal, heat shock, viral and transcription regulatory proteins contain single O-linked N-acetylglucosamine (O-GlcNAc) residues attached to serine or threonine residues. It has been observed that O-GlcNAc glycosylated proteins tend to be 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



Immunocytochemistry/ Immunofluorescence - Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739)

ab2739 stained in MCF7 cells. Cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab2739 at 5µg/ml and ab6046 (Rabbit polyclonal to beta Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150080 (pseudo-colored red) and ab150117 (colored green) used at 1 ug/ml for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739)

All lanes : Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739) at 1 µg/ml

Lanes 1 & 3 : Jurkat cells treated with 0 uM PugNAc
Lane 2 : Jurkat cells treated with 50 uM PugNAc (3 hours)
Lane 4 : Jurkat cells treated with 4 mM glucosamine and 50 uM PugNAc (3 hours)

Lysates/proteins at 20 µg per lane.

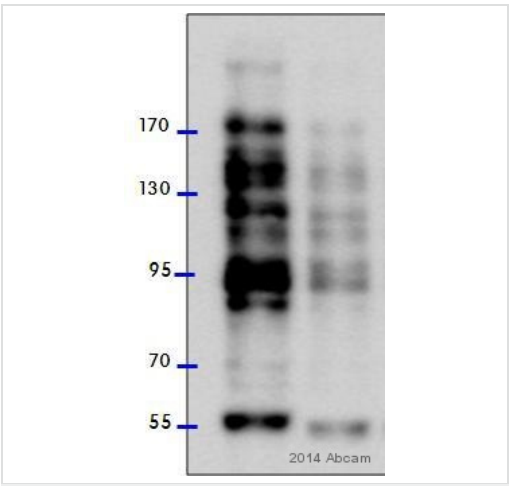
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 12 minutes

Jurkat cells were treated with either 50 uM PugNAc (ab144670) or 4 mM glucosamine + 50 uM PugNAc (ab144670) for three hours prior to harvest to stimulate O-linked glycosylation. The expected increase in glycosylation is observed in the treated lanes 2 & 4.



Western blot - Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739)

This image is courtesy of an anonymous Abreview

All lanes : Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739) at 1/3000 dilution

Lane 1 : Human neuroblastoma (SH-SY5Y) whole cell lysate - treated with 50µM z-Pugnac for 24 hours
Lane 2 : Human neuroblastoma (SH-SY5Y) whole cell lysate - untreated

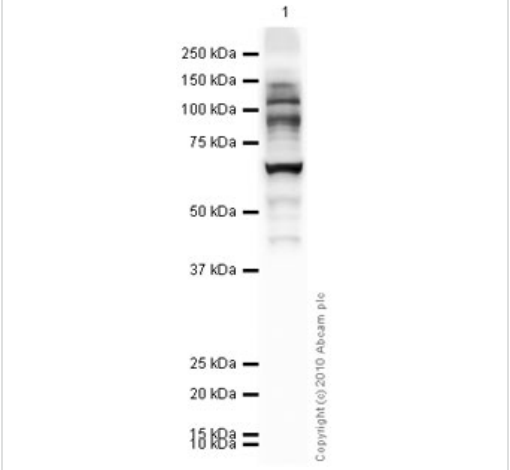
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP-conjugated horse anti-mouse IgG polyclonal

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 30 seconds



Western blot - Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739)

Anti-O-Linked N-Acetylglucosamine antibody [RL2] (ab2739) at 1 µg/ml + Rat Liver Nuclear Envelope at 10 µg

Secondary
Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 1 minute

The antibody was tested against the immunogen (isolated rat liver nuclear envelopes, which contain 8 O-linked glycoproteins in the nuclear pore complex).

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