Product Datasheet

O-GlcNAc Antibody (RL2) - BSA Free
NB300-524

Unit Size: 0.1 ml

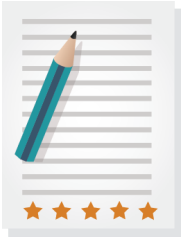
Store at -20C. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Reviews: 2 Publications: 30

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB300-524

Updated 10/23/2024 v.20.1



NB300-524

O-GlcNAc Antibody (RL2) - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	RL2
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein A purified
Buffer	PBS

Product Description

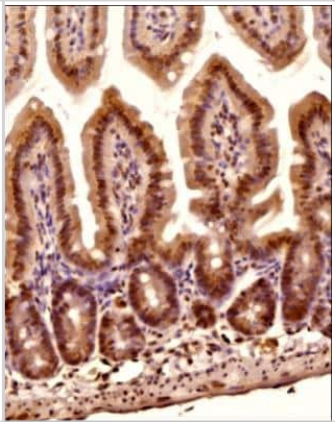
Host	Mouse
Species	Human, Mouse, Rat, Porcine, Bovine, Drosophila, Fish, Hamster, Primate, Virus, Xenopus
Reactivity Notes	Porcine reactivity reported in scientific literature (PMID: 26004176). Xenopus reactivity reported in scientific literature (PMID: 17329255). Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information.
Specificity/Sensitivity	Detects nuclear pore complex (NPC), cytoplasmic and intranuclear O-linked glycoproteins from human, mouse, and rat tissues.
Immunogen	Pore complex-lamina fraction purified from rat liver nuclear envelopes.

Product Application Details

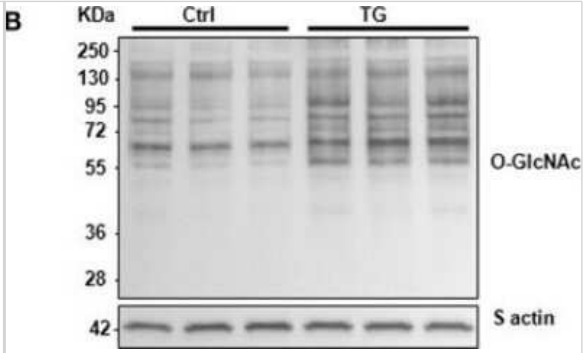
Applications	Western Blot, Chromatin Immunoprecipitation, Dot Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:1000, Chromatin Immunoprecipitation 1:10 - 1:500. Use reported in scientific literature (PMID 20404350), Flow Cytometry 1:10 - 1:1000, ELISA 1:100 - 1:2000. Use reported in scientific literature (PMID 12029848), Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:200, Dot Blot 1:800, Chromatin Immunoprecipitation (ChIP) 1:10-1:500

Images

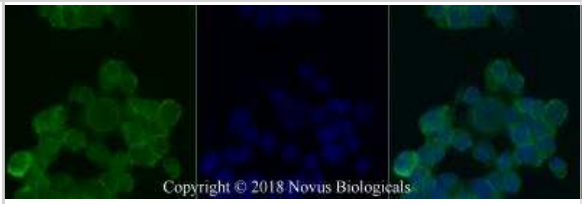
Immunohistochemistry-Paraffin: O-GlcNAc Antibody (RL2) [NB300-524] - Analysis of a FFPE tissue section of the mouse colon using 1:200 dilution of O-GlcNAc [RL2] antibody (NB300-524). The signal was developed using HRP-DAB method which followed counterstaining of the cells with hematoxylin.



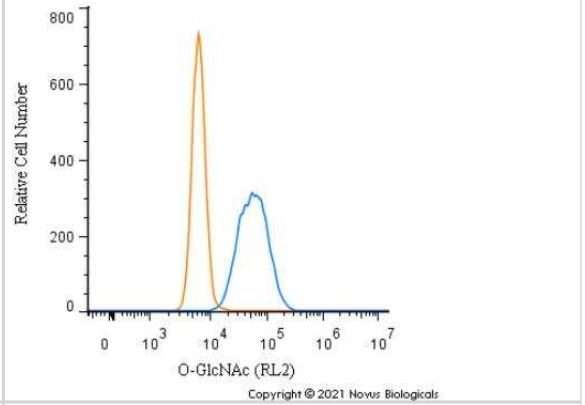
Western Blot: O-GlcNAc Antibody (RL2) [NB300-524] - Impact of OGA inhibition by thiamet G in primary cultures of NCM. Representative western blots (left panel) and quantification (right panel) of O-GlcNAcylated proteins levels in control (Ctrl) and NCM treated with 100 nM of thiamet G (TG) during 24 h (n = 12). Image collected and cropped by Citeab from the following publication (Interplay Between Phosphorylation and O-GlcNAcylation of Sarcomeric Proteins in Ischemic Heart Failure. Front Endocrinol (Lausanne) (2018) licensed under a CC-BY license.



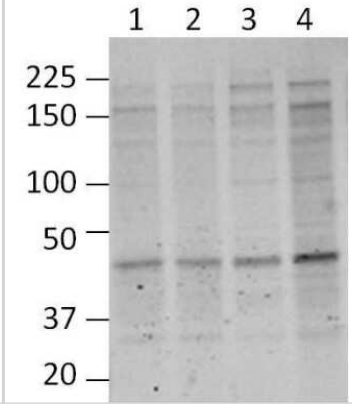
Immunocytochemistry/Immunofluorescence: O-GlcNAc Antibody (RL2) [NB300-524] - Neuro2a cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-O-GlcNAc (RL2) at 5 ug/mL overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



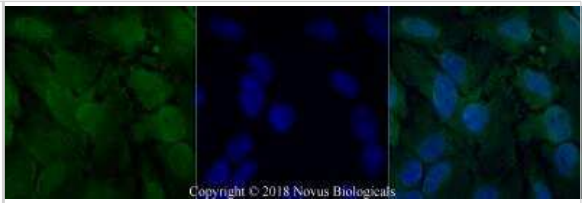
Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on Neuro2a cells with O-GlcNAc Antibody [RL2] NB300-524 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).



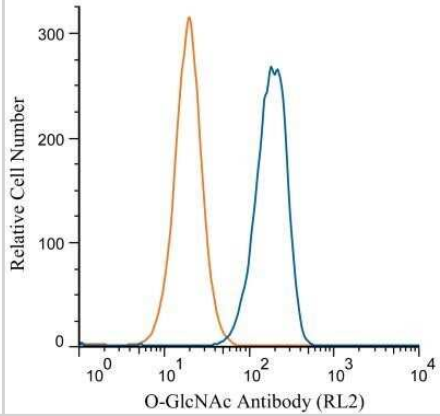
Western Blot: O-GlcNAc Antibody (RL2) [NB300-524] - Analysis of mouse cortical brain lysates using O-Linked N-Acetylglucosamine Monoclonal Antibody. Blots containing cortical extracts from 4 individual C57BL/6 mice (Lanes 1-4) were blocked with 5% milk in TBST, and probed with MA1-072 at 1:1000, followed by a fluorophore-conjugated goat anti-mouse IgG secondary antibody. Data courtesy of the Innovators Program.



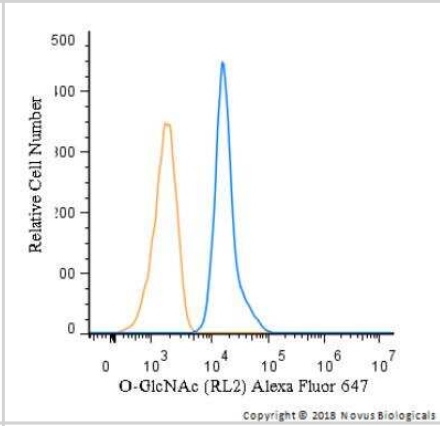
Immunocytochemistry/Immunofluorescence: O-GlcNAc Antibody (RL2) [NB300-524] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-O-GlcNAc (RL2) at 5 ug/mL overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



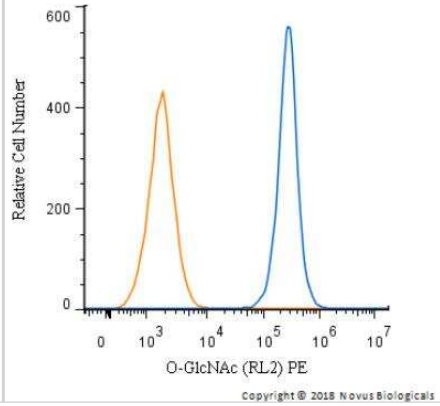
Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - Analysis using Alexa Fluor (R) 647 conjugate of NB300-524. An intracellular stain was performed on Jurkat cells with O-GlcNAc antibody (RL2) NB300-524 (blue) and a matched isotype control NBP2-27287 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. 1 ug of antibody was added to 100 uL of staining buffer and cells were incubated for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.

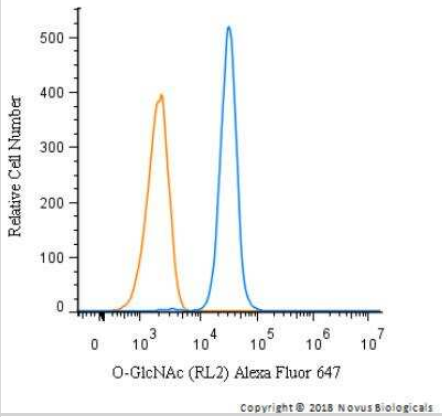
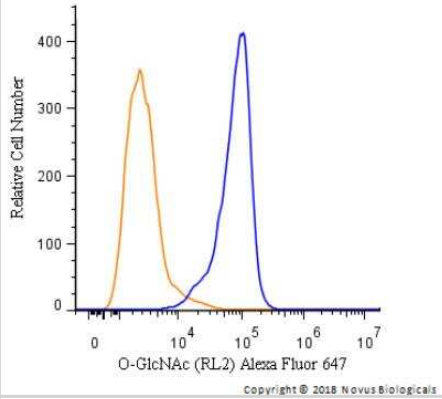
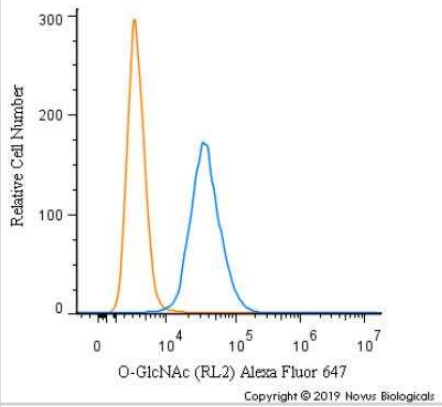
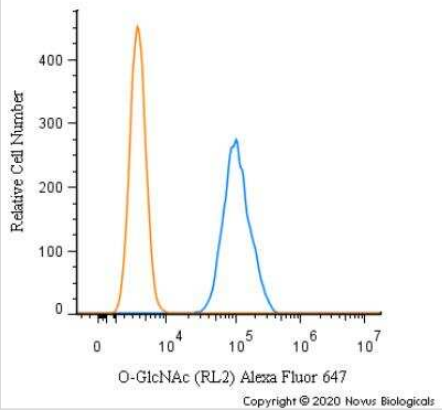


Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on U-937 cells with O-GlcNAc antibody (RL2) NB300-524AF647 (blue) and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature . Both antibodies were conjugated to Alexa Fluor 647.

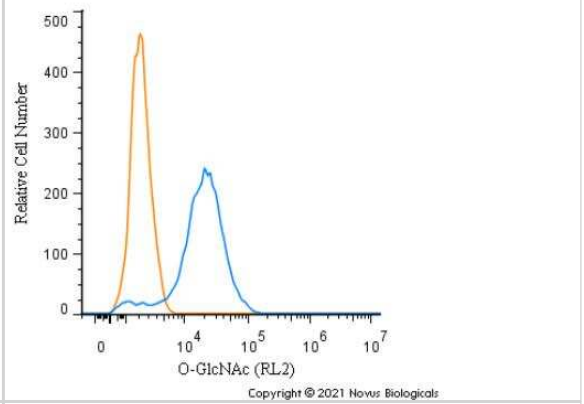


Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on Jurkat cells with O-GlcNAc antibody (RL2) NB300-524PE (blue) and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature . Both antibodies were conjugated to Phycoerythrin.

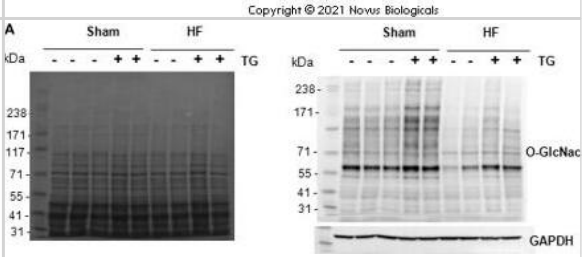


Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on SK-MEL-28 cells with O-GlcNAc antibody (RL2) NB300-524AF647 (blue) and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature . Both antibodies were conjugated to Alexa Fluor 647.	
Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on HeLa cells with O-GlcNAc Antibody [RL2] Antibody NB300-524AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on Neuro2a cells with O-GlcNAc Antibody [RL2] NB300-524AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on RH30 cells with O-GlcNAc [RL2] Antibody NB300-524AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	

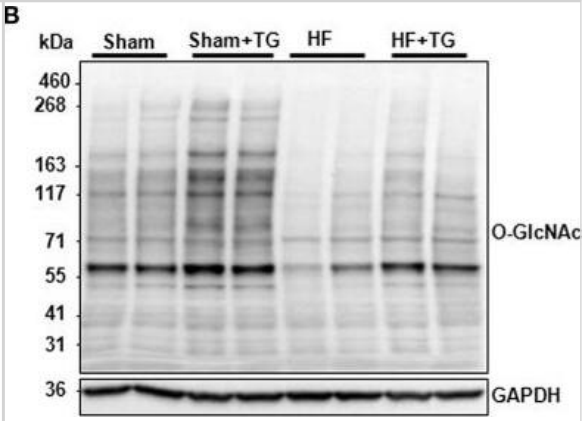
Flow Cytometry: O-GlcNAc Antibody (RL2) [NB300-524] - An intracellular stain was performed on Jurkat cells with O-GlcNAc Antibody [RL2] NB300-524 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).



Western Blot: O-GlcNAc Antibody (RL2) - BSA Free [NB300-524] - Analysis of O-GlcNAcylated LV proteins by Western blot & WGA-SDS-PAGE gel electrophoresis. (A) Red ponceau staining (left panel) & western blot (right panel) of O-GlcNAcylated proteins (50 µg) extracted from sham- & HF-rats treated or not with thiamet G. The positions of molecular weight are indicated as kilodalton (kDa) on the left. (B) Red ponceau staining (left panel) & WGA-SDS-PAGE of O-GlcNAcylated proteins levels (middle panel) of O-GlcNAcylated desmin levels (right panel) from the same samples. The arrow in desmin WGA gels indicates the non-O-GlcNAcylated form. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30344511), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: O-GlcNAc Antibody (RL2) - BSA Free [NB300-524] - Effect of OGA inhibition by thiamet G in isolated perfused heart. (A) Description of the protocol designed for thiamet G (TG) perfusion in sham- (n = 6) & HF- (n = 7) rats 6 weeks post-MI. (B) Western blot (left panel) & quantification (right panel) of O-GlcNAcylated proteins levels measured in proteins extracted from LVs of isolated perfused sham- & HF-rat hearts treated or not with 100 µM thiamet G for 2 h (n = 7 in each group). (C) Western blots (upper panel) & quantification (lower panel) of total desmin levels in the same samples. (D) Phosphorylation profiles of desmin were analyzed in the same samples by Phos-tag™ gel. Graphs show mean ± SEM values expressed in arbitrary units (A.U.). The positions of molecular weight are indicated as kilodalton (kDa) on the left. *P < 0.05; ** < 0.01. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30344511), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Florence Authier, Nina Ondruskova, Andrew T Ferenbach, Alison McNeilly, Daan M F van Aalten Neurodevelopmental defects in a mouse model of O-GlcNAc transferase intellectual disability. Disease models & mechanisms 2024-04-03 [PMID: 38566589]

Authier F, Attianese B, Bartual S et al. Intellectual disability and neurogenesis defects associated with increased turnover of an O-GlcNAcase variant medRxiv 2023-11-24 (WB)

Kadosaka T, Watanabe M, Natsui H et al. Empagliflozin attenuates arrhythmogenesis in diabetic cardiomyopathy by normalizing intracellular Ca2+ handling in ventricular cardiomyocytes American journal of physiology. Heart and circulatory physiology 2023-03-01 [PMID: 36607794]

Murray M, Davidson L, Ferenbach A et al. Neuroectoderm phenotypes in a human stem cell model of O-GlcNAc transferase intellectual disability bioRxiv 2023-09-21 (WB, Human)

Details:
1:1000 dilution

Authier F, Ondruskova N, Ferenbach A et al. Neurodevelopmental defects in a mouse model of O-GlcNAc transferase intellectual disability bioRxiv 2023-08-24 (WB, Mouse)

Feng Z, Wang T, Sun Y et al. Sulforaphane suppresses paraquat-induced oxidative damage in bovine□in vitro-matured oocytes through Nrf2 transduction pathway Ecotoxicology and environmental safety 2023-04-01 [PMID: 36907095] (Western Blot, Bovine)

Czajewski I, McDowall L, Ferenbach A Et al Rescuable sleep and synaptogenesis phenotypes in a Drosophila model of O-GlcNAc transferase intellectual disability bioRxiv 2023-06-30 (WB)

Omelková M, Fenger CD, Murray M et al. An O-GlcNAc transferase pathogenic variant linked to intellectual disability affects pluripotent stem cell self-renewal Disease models & mechanisms 2023-06-01 [PMID: 37334838] (WB, Mouse)

Pelgrom LR, Sergushichev AA, Quik M Protein O-GlcNAcylation and low glycolysis underpin Th2 polarization by dendritic cells Book 2022-01-01 (WB, Mouse)

Jo R, Shibata H, Kurihara I et al. Mechanisms of mineralocorticoid receptor-associated hypertension in diabetes mellitus: the role of O-GlcNAc modification Hypertension research : official journal of the Japanese Society of Hypertension 2022-10-14 [PMID: 36229526]

Ise H, Araki Y, Song I, Akatsuka G N-acetylglucosamine-bearing polymers mimicking O-GlcNAc-modified proteins elicit anti-fibrotic activities in myofibroblasts and activated stellate cells Glycobiology 2022-10-03 [PMID: 36190502]

Kumar A, Yarosz EL, Andren A et al. NKT cells adopt a glutamine-addicted phenotype to regulate their homeostasis and function Cell reports 2022-10-25 [PMID: 36288696]

More publications at http://www.novusbio.com/NB300-524

Procedures

Immunohistochemistry-Paraffin protocol for O-GlcNAc Antibody (NB300-524)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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Products Related to NB300-524

NBL1-13919	O-GlcNAc Transferase p110 subunit Overexpression Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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