O-GlcNAc Monoclonal Antibody (RL2), PE-Cyanine7, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	All, Human
Host/Isotope	Mouse / IgG1, kappa
Recommended Isotype Control	Mouse IgG1 kappa Isotype Control (P3.6.2.8.1), PE-Cyanine7, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	RL2
Conjugate	PE-Cyanine7
Immunogen	Rat Liver nuclear envelopes
Form	Liquid
Concentration	5 µl/Test
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2, with 0.2% BSA, 0.1% gelatin
Contains	0.09% sodium azide
Storage Conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2744718

Applications	Tested	Dilution	Published
Flow Cytometry (Flow)	✓	5 µL (0.25 µg)/test	

Product Specific Information

Description: This RL2 monoclonal antibody recognizes proteins with O-N-acetylglucosamine (O-GlcNAc) glycosylation. It was originally developed by immunizing mice with the rat liver nuclear envelopes containing nuclear pore complexes. The RL2 clone has been successfully used in Western blot, immunofluorescence, immunoprecipitation, and flow cytometry in a wide variety of mammalian cells.

Applications Reported: This RL2 antibody has been reported for use in flow cytometric analysis.

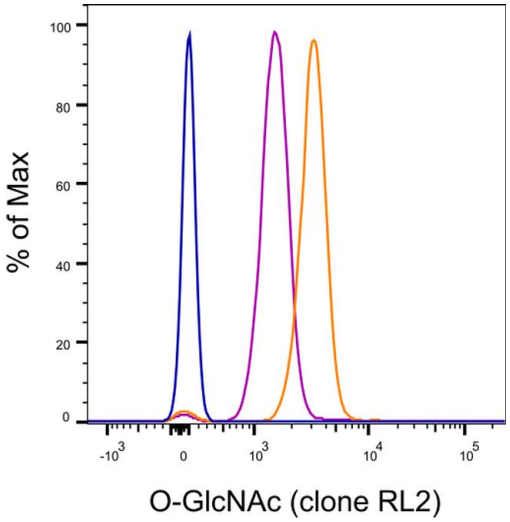
Applications Tested: This RL2 antibody has been pre-diluted and tested by flow cytometric analysis of normal human peripheral blood cells using the Foxp3/Transcription Factor Staining Buffer Set (Product # 00-5523) and protocol. Please refer to Best Protocols: Protocol B: One step protocol for (nuclear) intracellular proteins located under the Resources Tab online. This may be used at 5 µL (0.25 µg) per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test.

Light sensitivity: This tandem dye is sensitive to photo-induced oxidation. Please protect this vial and stained samples from light.

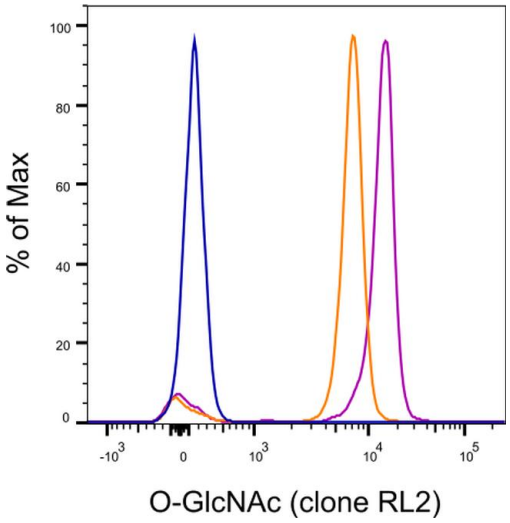
Fixation: Samples can be stored in IC Fixation Buffer (Product # 00-8222) (100 µL of cell sample + 100 µL of IC Fixation Buffer) or 1-step Fix/Lyse Solution (Product # 00-5333) for up to 3 days in the dark at 4°C with minimal impact on brightness and FRET efficiency/compensation. Some generalizations regarding fluorophore performance after fixation can be made, but clone specific performance should be determined empirically.

Excitation: 488-561 nm; Emission: 775 nm; Laser: Blue Laser, Green Laser, Yellow-Green Laser

Advanced Verification Data

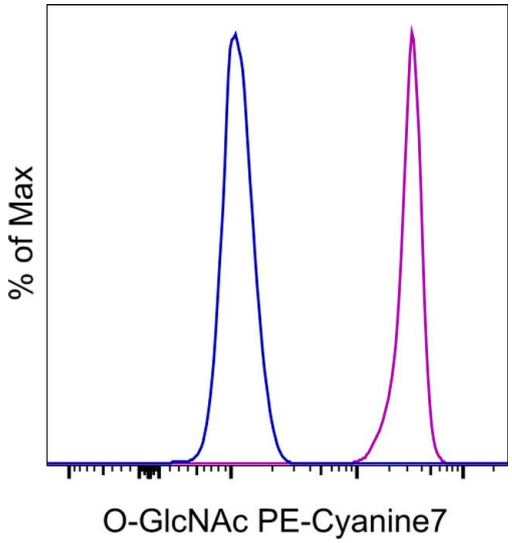


O-GlcNAc Antibody (25-9793-42)
Intracellular staining of human peripheral blood cells treated with OSMI-1, an inhibitor of O-GlcNAc transferase (OGT). As expected, O-GlcNAc clone RL2 stains cells with diminished intensity when the addition of O-GlcNAc is blocked by OSMI-1, compared with untreated cells. Details: Normal human peripheral blood cells were cultured for 4 hours with OSMI-1 (50 µM) (purple histogram) or without it (orange histogram). Cells were then fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Set and protocol prior to intracellular staining with O-GlcNAc (clone RL2) (purple and orange histograms) or mouse IgG1 kappa Isotype control (blue histogram). Cells in the lymphocyte gate were used for analysis. Cell Treatment validation info.



O-GlcNAc Antibody (25-9793-42)
Intracellular staining of human peripheral blood cells treated with Thiamet-G, an inhibitor of O-GlcNAc hydrolase. As expected based on published data, O-GlcNAc clone RL2 stains cells with increased intensity when the removal of O-GlcNAc is blocked with Thiamet-G compared with untreated cells. Details: Normal human peripheral blood cells were cultured overnight with Thiamet-G (100nM) (purple histogram) or without it (orange histogram). Cells were stained with a fixable viability dye and fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Set and protocol prior to intracellular staining with O-GlcNAc (clone RL2) (purple and orange histograms) or mouse IgG1 kappa Isotype control (blue histogram). Cells in the lymphocyte gate were used for analysis. Cell Treatment validation info.

Product Images For O-GlcNAc Monoclonal Antibody (RL2), PE-Cyanine7, eBioscience™



O-GlcNAc Antibody (25-9793-42) in Flow
Normal human peripheral blood cells were stained intracellularly, using the Foxp3 /Transcription Factor Staining Buffer Set (Product # 00-5523-00) and protocol, with Mouse IgG1 kappa Isotype Control, PE-Cyanine7 (Product # 25-4714-82) (blue histogram) or O-GlcNAc Monoclonal Antibody, PE-Cyanine7 (purple histogram). Cells in the lymphocyte gate were used for analysis.