

Product Datasheet

Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary Antibody (eB182) [DyLight 800] NBP3-11666

Unit Size: 100 ul

Store lyophilized antibody at 4C. Aliquot reconstituted liquid and store at -20C. Avoid freeze-thaw cycles.

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NBP3-11666

Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary Antibody (eB182) [DyLight 800]

Product Information	
Unit Size	100 ul
Concentration	LYOPH mg/ml
Storage	Store lyophilized antibody at 4C. Aliquot reconstituted liquid and store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	eB182
Preservative	0.01% Sodium Azide
Reconstitution Instructions	Reconstitute with 100 ul deionized water (or equivalent).
Isotype	IgG
Conjugate	DyLight 800
Purity	Protein G purified
Buffer	Lyophilized from 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 10 mg/ml Polyethylene Glycol (PEG-8000)

Product Description	
Description	<p>Store vial at 4C prior to restoration. For extended storage aliquot contents and freeze at -20C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4C as an undiluted liquid. Dilute only prior to immediate use.</p> <p>This product was prepared from tissue culture supernatant by Protein G affinity chromatography. Assay by Immunoelectrophoresis resulted in a single precipitin arc against Anti-Rabbit Serum</p>
Host	Mouse
Species	Rabbit
Specificity/Sensitivity	Reactivity is observed against native Rabbit IgG by both Western blot and ELISA.
Immunogen	Rabbit IgG

Product Application Details	
Applications	Western Blot, Fluorophore-linked immunosorbent assay, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry 1:2000 - 1:10000, Immunohistochemistry, Immunocytochemistry/Immunofluorescence 1:500 - 1:2500, Immunoprecipitation, Fluorophore-linked immunosorbent assay



Application Notes

This secondary antibody may also be used for detection in immunoassays that do not employ immunoprecipitation. It is provided as a lyophilized powder. To conserve reagent, we recommend incubating the blots from minigels in sealed bags (removing as much air as possible) with minimal volume (2-3 mLs). If used conservatively at 2.5mLs/blot will yield enough reagent for 200 blots.

Note that there are three key procedural considerations:

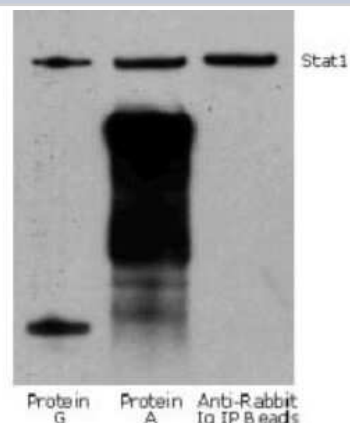
1. Protein A or G should not be used for the immunoprecipitation. Use of protein A or G beads with the rabbit Pure-Blot will result in contaminating bands. For immunoprecipitation, Anti-rat IgG beads, or Anti-rabbit IgG beads should be used for rat or rabbit immunoprecipitating antibodies, respectively.
2. Immunoprecipitate should be completely reduced.
3. Bovine Serum Albumin, or blocking buffer for fluorescent western blotting, at low concentrations, should be used as the blocking protein for the immunoblot. **DO NOT USE BLOTTO or MILK.** All recommended dilutions for listed applications are intended as an initial recommendation, specific conditions for each protein and antibody combination should be specifically optimized by the end user.

Fluorescence technology is widely used to detect proteins. However, many common visible fluorophores often result in considerable background fluorescence in the visible range. Visible fluorophores are rarely used for membrane-based protein detection because of this high background. DyLight (TM) 800 and DyLight(TM) 680 antibody and reagent conjugates are specifically designed for protein detection methods that use longer-wavelength, near-infrared (IR) fluorophores to visualize proteins in western blotting and other applications. Very low background fluorescence in the IR range provides for a much higher signal-to-noise ratio than visible fluorophores. Detection levels in the picogram range on Western blots rival the sensitivity of chemiluminescence on film. DyLight(TM) 800 conjugates are also suitable for immunofluorescence microscopy using commercially available excitation/emission filters in the 780nm/820nm range. Dual simultaneous labeling in western blots or microscopy is achieved when DyLight(TM) 800 conjugates are used in conjunction with DyLight(TM) 680 conjugates. DyLight(TM) 800 and DyLight(TM) 680 conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging.

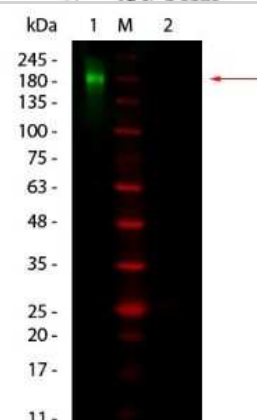


Images

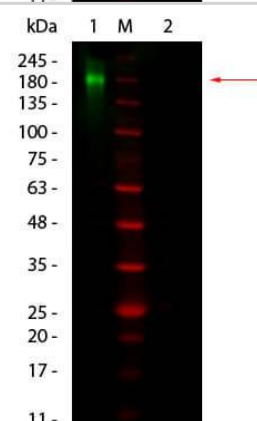
Western Blot: Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary Antibody (eB182) [DyLight 800] [NBP3-11666] - Jurkat cell lysate (0.5 ml of 1×10^7 cells/ml) was incubated with rabbit anti-human Stat1 and immunoprecipitated using Protein G, Protein A and Anti-Rabbit Ig IP Beads. Precipitate from 5×10^5 cells was subjected to electrophoresis, transferred to a PVDF membrane, and Western blotted with Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary antibody (eB182) [DyLight 800].



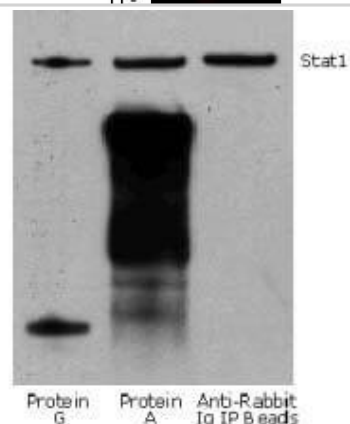
Western Blot: Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary Antibody (eB182) [DyLight 800] [NBP3-11666] - Lane 1: Rabbit IgG, Non-reduced. M: Opal Pre-stained Ladder. Lane 2: Rabbit IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary antibody (eB182) [DyLight 800] at 1:1,000 for 60 min at RT. Block for 30 min at RT. Predicted/Observed size: ~160 kDa for Rabbit IgG, Non-reduced.



Western Blot of Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary Antibody (eB182) [DyLight 800]. Lane 1: Rabbit IgG, Non-reduced. M: Opal Pre-stained Ladder



Mouse Pure-Blot anti-Rabbit IgG (H+L) Secondary Antibody (eB182) [DyLight 800] / Western Blot: Jurkat cell lysate (0.5 ml of 1×10^7 cells/ml) was incubated with rabbit anti-human Stat1 and immunoprecipitated using Protein G, Protein A and Anti-Rabbit Ig IP Beads. Precipitate from 5×10^5 cells was subjected to electrophoresis, transferred to a PVDF membrane, and Western blotted with anti-Stat1 using Rabbit TrueBlot (R): Anti-Rabbit IgG HRP





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Limitations

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

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