

Product Datasheet

Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC] NBP3-11662

Unit Size: 100 ul

Store at -4C in powder form. Store at -20C once reconstituted.

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NBP3-11662

Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC]

Product Information	
Unit Size	100 ul
Concentration	LYOPH mg/ml
Storage	Store at -4C in powder form. Store at -20C once reconstituted.
Clonality	Monoclonal
Clone	eB144
Preservative	0.01% Sodium Azide
Reconstitution Instructions	Reconstitute with 100 ul deionized water (or equivalent).
Isotype	IgG
Conjugate	FITC
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>Prepared from tissue culture supernatant by Protein G affinity chromatography. Assay by Immunoelectrophoresis resulted in a single precipitin arc against anti-fluorescein and Anti-Mouse Serum.</p>
Host	Rat
Species	Mouse
Specificity/Sensitivity	Reactivity is observed against native Mouse IgG by both Western blot and ELISA.
Immunogen	Mouse IgG

Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Immunocytochemistry/Immunofluorescence 1:500 - 1:2500, Immunoprecipitation



Application Notes

This secondary antibody may also be used for detection in immunoassays that do not employ immunoprecipitation. Fluorescein Conjugated Antibodies are designed for immunofluorescence microscopy, fluorescence based plate assays (FLISA) and fluorescent western blotting. This product is also suitable for multiplex analysis, including multicolor imaging, utilizing various commercial platforms.

This secondary antibody 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 40 blots.

Note that there are three key procedural considerations:

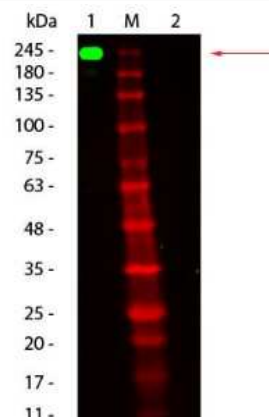
1. Protein A or G beads may be used with the mouse, goat and sheep Pure-Blot secondaries, but not with the rabbit Pure-Blot secondary. Use of protein A or G beads with the rabbit Pure-Blot will result in contaminating bands.
2. Immunoprecipitate should be completely reduced.
3. A blocking Buffer for fluorescent western blotting should be used as the blocking protein for the immunoblot. Note: To achieve best results when detecting mouse IgG1 subtypes, we recommend performing a dot blot or titration to determine the optimal dilution factor for your desired application. 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.

Suggested application: FISH

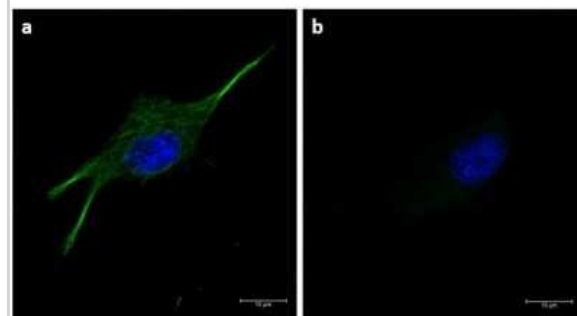


Images

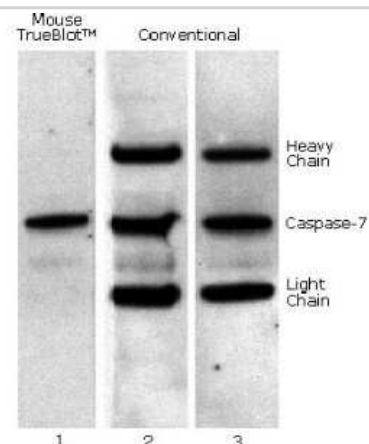
Western Blot: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC] [NBP3-11662] - Western Blot of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC]. Lane 1: Mouse IgG, Non-reduced. Lane 2: Mouse IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC] at 1:1,000 for 60 min at RT. Block for 30 min at RT. Predicted/Observed size: 160 kDa for Mouse IgG, Non-reduced. Migrates at slightly higher molecular weight. Other band(s): none.



Immunocytochemistry/Immunofluorescence: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC] [NBP3-11662] - U87-MG cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100) for 1hr, then incubated with 15ug/mL of anti-alpha-tubulin primary antibody at 4 degrees C overnight. Following 3 washes in 1X PBS for 5min each, 5ug/mL of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary antibody (eB144) [Fluorescein] was added and allowed to incubate for 1hr at room temperature. 5ug/mL of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary antibody (eB144) [FITC] was added and allowed to incubate for 1hr at room temperature. Nucleus was counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged alpha-tubulin (green)/DAPI (blue) image shown (b) secondary only.



Western Blot: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [FITC] [NBP3-11662] - Caspase 7 was immunoprecipitated from 0.5 ml of 1×10^7 Jurkat cells/ml with 5 ug mouse anti-human Caspase 7. Precipitate from 1×10^6 cells was subjected to electrophoresis, transferred to an PVDF membrane, and Western blotted with anti-Caspase 7 using Rat Pure-Blot anti-Mouse IgG (H+L) Secondary antibody (eB144) [FITC] (Lane 1) or conventional HRP-conjugated anti-mouse antibody (Lane 2) - note the detection of the heavy and light chains of the immunoprecipitating antibody in Lane 2 but not in Lane 1. When Lane 1 is re-immunoblotted using conventional HRP-conjugated anti-mouse polyclonal antibody (Lane 3), the heavy and light chains are now detected, confirming that although the immunoprecipitating heavy and light chains are present, Rat Pure-Blot anti-Mouse IgG (H+L) Secondary antibody (eB144) [FITC] detects only native antibody and not denatured heavy and light chains.





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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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