

Product Datasheet

Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] NBP3-11664

Unit Size: 100 ul

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

www.novusbio.com



technical@novusbio.com

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP3-11664

Updated 9/19/2023 v.20.1

Earn rewards for product
reviews and publications.

Submit a publication at www.novusbio.com/publications

Submit a review at www.novusbio.com/reviews/destination/NBP3-11664



NBP3-11664

Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680]

| 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 | eB144 |
| Preservative | 0.01% Sodium Azide |
| Reconstitution Instructions | Reconstitute with 100 ul deionized water (or equivalent). |
| Isotype | IgG |
| Conjugate | DyLight 680 |
| 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-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 |
| Notes | DyLight (R) is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries. |

| 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

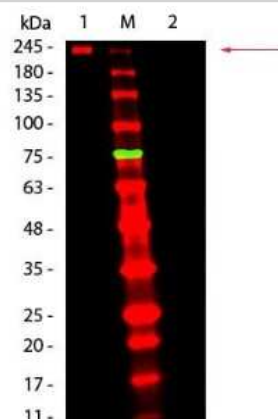
This secondary antibody may also be used for detection in immunoassays that do not employ immunoprecipitation. It is provided as a lyophilized powder. To conserve reagent, we recommend incubating the blots from minigels in sealed bags (removing as much air as possible) with minimal volume (2-3 mLs). If used conservatively at 2.5mls/blot will yield enough reagent for 40 blots.

Note that there are three key procedural considerations:

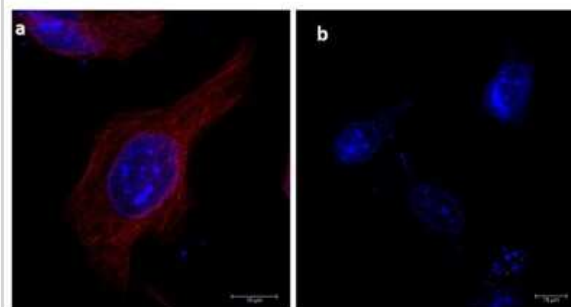
1. Protein A or G beads may be used with the mouse, goat and sheep Pure-Blot secondaries, but not with the rabbit Pure-Blot secondary. Use of protein A or G beads with the rabbit Pure-Blot will result in contaminating bands.
2. Immunoprecipitate should be completely reduced.
3. Blocking Buffer for fluorescent 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.

Images

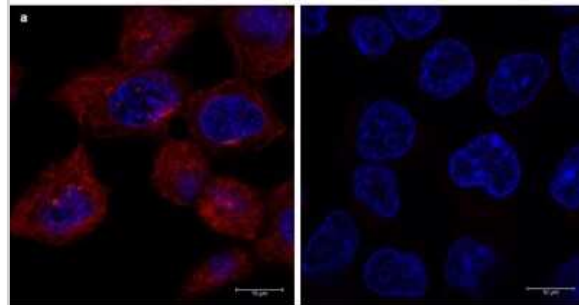
Western Blot: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] [NBP3-11664] - Western Blot of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680]. Lane 1: Mouse IgG, Non-reduced. Lane 2: Mouse IgG, Reduced. Load: 50 ng per lane. Primary antibody: none. Secondary antibody: Mouse Pure-Blot anti-Rabbit IgG Secondary antibody (eB144) [DyLight 680] 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.



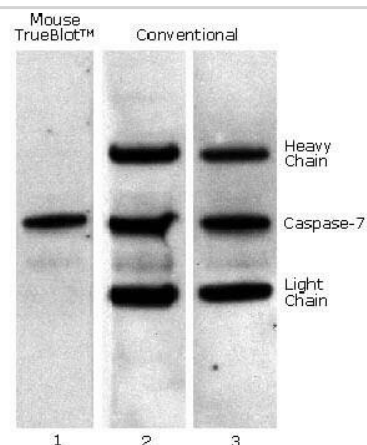
Immunocytochemistry/Immunofluorescence: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] [NBP3-11664] - Immunofluorescence microscopy of alpha-tubulin in HeLa cells using Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] for detection. HeLa cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100 in 1X PBS) for 1hr, then incubated with 15ug/mL of anti-alpha-tubulin primary antibody at 4C overnight. Following 3 washes in 1X PBS for 5 min each, 5ug/mL of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] was added and allowed to incubate for 1hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged alpha-tubulin (red)/DAPI (blue) image shown. (b) secondary antibody only.



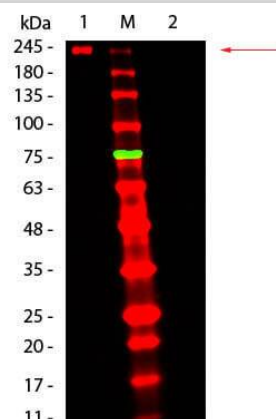
Immunocytochemistry/Immunofluorescence: Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] [NBP3-11664] - Immunofluorescence microscopy of alpha-tubulin in A431 cells using Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] for detection. A431 cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100 in 1X PBS) for 1 hr, then incubated with 15 ug/mL of anti-alpha-tubulin primary antibody at 4 degrees C overnight. After 3 washes in 1X PBS for 5 min each, 5 ug/mL of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] was added and allowed to incubate for 1 hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged alpha-tubulin (red)/DAPI (blue) image shown. (b) secondary antibody only.



Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] / Western Blot: 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 Mouse TrueBlot(R) ULTRA: Anti-Mouse Ig HRP (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, Mouse TrueBlot(R) ULTRA: Anti-Mouse Ig HRP detects only native antibody and not denatured heavy and light chains.



Western Blot of Rat Pure-Blot anti-Mouse IgG (H+L) Secondary Antibody (eB144) [DyLight 680] Conjugated. 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) [DyLight 680] Conjugated 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.





Novus Biologicals USA

10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada

21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd

19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information

www.novusbio.com
Technical Support: nb-technical@bio-
techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

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.

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

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NBP3-11664

Earn gift cards/discounts by submitting a publication using this product:
www.novusbio.com/publications

