Technical Data Sheet

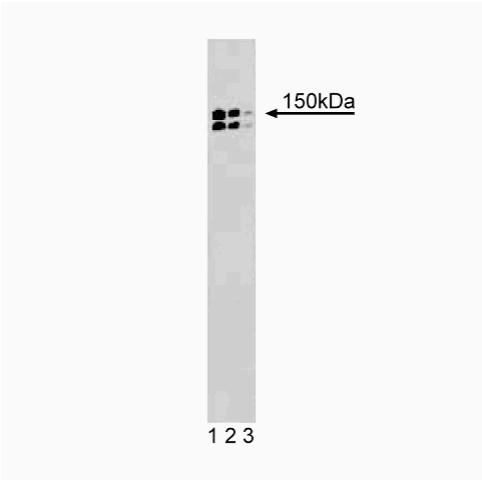
Purified Mouse Anti-p150 [Glued]

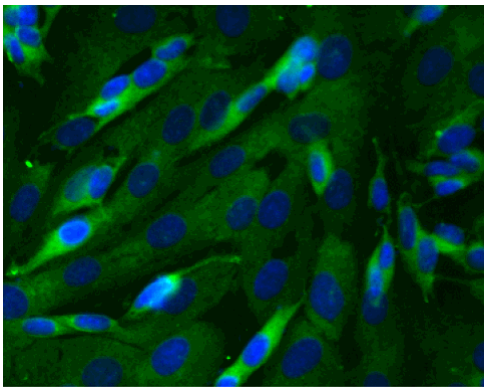
Product Information

Material Number:	**612708**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	12/P150GLUED
Immunogen:	Rat p150 [Glued] aa. 3-202
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Rat Tested in Development: Mouse, Human, Dog
Target MW:	150 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

p150 [Glued] was identified as a polypeptide associated with cytoplasmic dynein,the minus-end-directed microtubule-based motor protein. p150 [Glued] is also a member of the oligomeric dynactin complex. Dynactin mediates dynein-driven vesicle motility, as well as lower eukaryote nuclear transport. p150 [Glued] bears significant homology to the product of the Glued gene in Drosophila. It has been shown in vitro to be a required activator of dynein-mediated transport along microtubules. The p150 [Glued] component of the dynactin complex binds to microtubules and the actin-like protein Centractin (Arp-1), another member of the dynactin complex. In the developing rat, p150 [Glued] is expressed at high levels in neural tissue. Microtubule binding assays with selected constructs of p150 [Glued] indicate that amino acids 39-150 are required for microtubule association.





Western blot analysis of p150 [Glued] on a rat cerebrum lysate (left). *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the mouse anti- p150 [Glued] antibody.*

Immunofluorescent staining of SK-N-SH cells (Human neuroblastoma; ATCC HTB-11) (right). *Cells were seeded in collagen coated 384-well imaging microplates (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) with the mouse anti- p150 [Glued] antibody and counter-stained with Hoechst 33342 (pseudo-colored blue). The second step reagent used was Alexa Fluor® 488 goat anti-mouse Ig (pseudo-colored green) (Invitrogen). The images were captured on a BD Pathway™ 855 or 435 Bioimager system using a 20x objective and merged using BD Attovision™ software. This antibody also stained SH-SY5Y (Human neuroblastoma; ATCC CRL-2266) and C6 (Rat glioma; ATCC CCL-107) cells using both the Triton-X 100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Bioimaging: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Bioimaging_Certified.shtml

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611463	Rat Cerebrum Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353962	BD Falcon™ 384-well Imaging Plate	1 box	test clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Holzbaur EL, Hammarback JA, Paschal BM, Kravit NG, Pfister KK, Vallee RB. Homology of a 150K cytoplasmic dynein-associated polypeptide with the Drosophila gene Glued. 1991; 351(6327):579-583.(Biology)

Waterman-Storer CM, Karki S, Holzbaur EL. The p150Glued component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp-1). 1995; 92(5):1634-1638.(Biology)