Technical Data Sheet

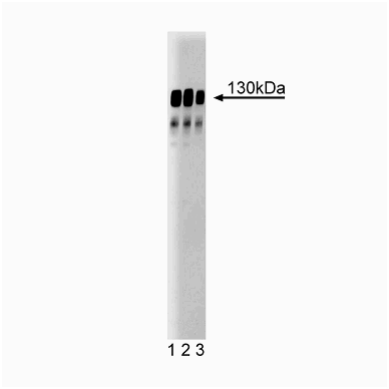
Purified Mouse Anti-GRIP

Product Information

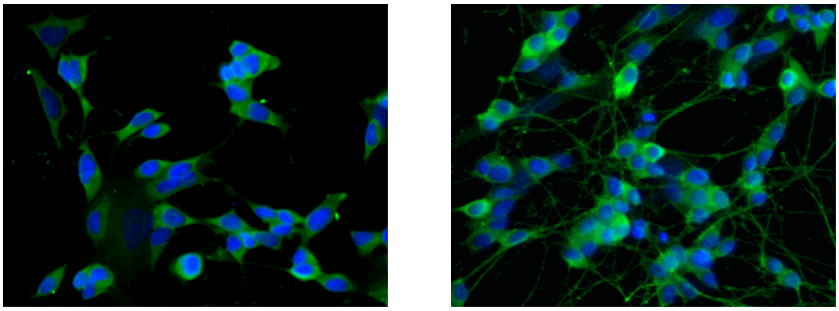
Material Number:	**611318**
Alternate Name:	Glutamate Receptor Interacting Protein
Size:	50 µg
Concentration:	250 µg/ml
Clone:	32/GRIP
Immunogen:	Rat GRIP aa. 877-1067
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Rat
Target MW:	130 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Rapid neuronal excitation within the CNS is mediated by the interactions of receptors with their respective neurotransmitters, such as glutamate. Glutamate has a diverse array of receptors that are categorized into two distinct groups: ionotropic and metabotropic. Ionotropic receptors are ligand-gated channels and can be subdivided into two classes: NMDA and AMPA receptors. AMPA receptors are composed of four homologous subunits (GluR1-4) that differentially combine to form a variety of receptor subtypes. Interactions of the subunits with cytoplasmic proteins mediate the transmission of extracellular signals. GRIP (Glutamate Receptor Interacting Protein) specifically interacts with the C-terminus of the GluR2 subunit. GRIP lacks a catalytic domain, but contains seven PDZ domains which are motifs that mediate protein-protein interactions. Since only the fourth and fifth PDZ domains are utilized in interaction with GluR2, it is thought that the remaining PDZ domains interact with other unidentified proteins. Therefore, GRIP functions as an adaptor that links AMPA receptors to cytoskeletal and/or signaling molecules and, in turn, clusters them at the synapse.



Western blot analysis of GRIP on a rat cerebrum lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the mouse anti-GRIP antibody.*



Immunofluorescence staining of undifferentiated SH-SY5Y cells (Human neuroblastoma; ATCC CRL-2266) (left) and differentiated SH-SY5Y cells (right). *Undifferentiated cells were seeded in a collagen coated 384-well imaging plate (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the mouse anti-GRIP antibody. Differentiated cells were seeded in a 96-well, collagen coated imaging plate (Material # 353219) at ~ 5,000 cells per well. Cells were incubated with 50 mM ATRA (Sigma-Aldrich, cat.no. R2625) for 5 days, followed by 50 ng/ml BDNF (Sigma-Aldrich, cat.no. B3795) for 5 days. Differentiated cells were fixed and stained using the methanol fix/perm protocol, and the mouse anti-GRIP antibody. The second step reagent in both cases was Alexa Fluor® 488 goat anti-mouse Ig (Invitrogen). The images were taken on a BD Pathway™ 855 or 435 Bioimager using a 20x objective. This antibody also stained undifferentiated SK-N-SH (Human neuroblastoma; ATCC HTB-11), C6 (Rat glioma; ATCC CCL-107), U-87 MG (Human glioblastoma cells; ATCC HTB-14) and U-373 cells (Human glioblastoma cells; ATCC HTB-17; discontinued, investigators may refer to: http://www.atcc.org/MisidentifiedCellLines/tabid/683/Default.aspx) using both the Triton-X 100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

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

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:

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)
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
353962	BD Falcon™ 384-well Imaging Plate	1 box	test clone

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

Bowery NG, Brown DA. The cloning of GABA(B) receptors. *Nature.* 1997; 386(6622):223-224.(Biology)
Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Huganir RL. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature.* 1997; 386(6622):279-284.(Biology)
Fallon L, Moreau F, Croft BG, Labib N, Gu WJ, Fon EA. Parkin and CASK/LIN-2 associate via a PDZ-mediated interaction and are co-localized in lipid rafts and postsynaptic densities in brain. *J Biol Chem.* 2002; 277(1):486-491.(Biology: Western blot)
Setou M, Seog DH, Tanaka Y. Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature.* 2002; 417(6884):83-87.(Biology: Immunofluorescence, Immunoprecipitation, Western blot)