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Datasheet for 600-401-MP0S

Ephrin B1, B2, B3 Antibody

Overview

Description:	Anti-Ephrin B1, B2, B3 (RABBIT) Antibody - 600-401-MP0S
Item No.:	600-401-MP0S
Size:	25 µL
Applications:	ELISA, IF, WB
Reactivity:	Human, Mouse, Xenopus laevis
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI). Ephrin B1, B2, B3 are cell surface transmembrane ligands for Eph receptors, a family of receptor tyrosine kinases which are crucial for migration, repulsion and adhesion during neuronal, vascular and epithelial development. Binding to Eph receptors residing on adjacent cells leads to contact-dependent bidirectional signaling into neighboring. Ephrin B1 shows high affinity for the receptor tyrosine kinase EPHB1/ELK and can bind EPHB2 and EPHB3. Ephrin B3 may play a pivotal role in forebrain function. They binds to, and induces collapse of, commissural axons/growth cones in vitro. And may play a role in constraining the orientation of longitudinally projecting axons. Anti-Ephrin B1, B2, B3 Antibody is useful for researchers interested in Developmental Biology and Tyrosine Kinases/Adaptors.
Synonyms:	Rabbit Anti-Ephrin B1, B2, B3 Antibody, Ephrin-B1, Ephrin-B2, Ephrin-B3, ELK Ligand, EPLG2, LERK2, EFL3, Craniofrontonasal Syndrome (Craniofrontonasal Dysplasia), EPH-Related Receptor Transmembrane Ligand ELK-L3, Eph-Related Receptor Tyrosine Kinase Ligand 2, Eph-Related Receptor Tyrosine Kinase Ligand 8, EPH-Related Receptor Tyrosine Kinase Ligand 2, LERK-2, LERK-8, Elk-L, EFL-3, ELK-L, CFND, EFL6, EFB1, CFNS
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	EFNB1, Efnb2, EFNB3

Reactivity:	Human, Mouse, Xenopus laevis
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-Ephrin B1, B2, B3 antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to C-terminal portion of human Ephrin B1, B2, B3 conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	This affinity purified antibody is directed against human Ephrin B1, B2, B3. This product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest cross-reactivity with the antigen based on 100% homology with Ephrin-B1 to human, mouse, rat, and Gallus gallus; 100% homology with Ephrin-B2 to mouse; 100% homology with Ephrin-B3 to human and mouse.
Relevant Links:	• UniProtKB - P52800 • UniProtKB - P98172 • NCBI - NP_004420.1 • GeneID - 1947 • UniProtKB - Q15768

Application Details

Tested Applications:	ELISA, IF, WB
Application Note:	Anti-Ephrin B1, B2, B3 Antibody has been tested in ELISA, Western Blot, and Immunofluorescence. Expect a band at ~35-38 kDa in western blot using appropriate lysates or tissues. Positive control used Xenopus laevis and mouse kidney in WB and Xenopus laevis in IF.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 50,000
IF:	1:5000
WB:	1:1000

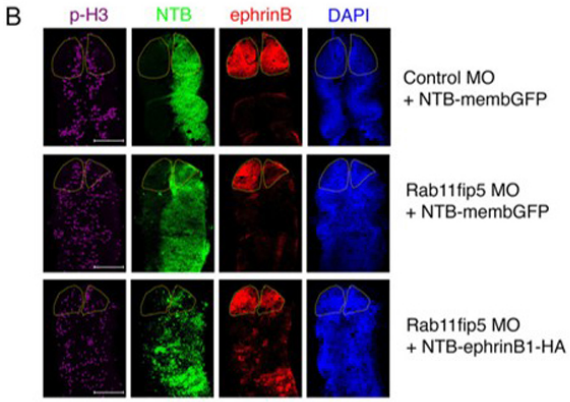
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.05 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

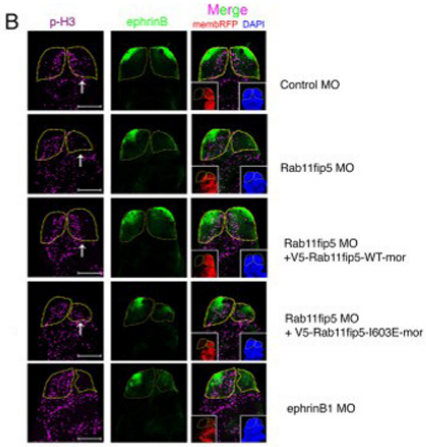
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

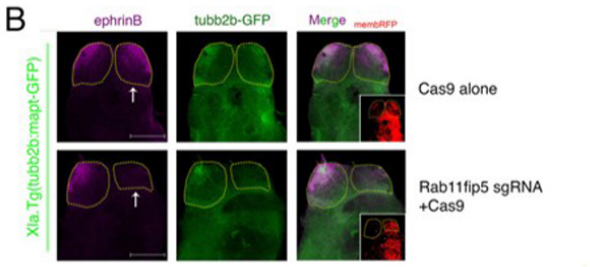
Images



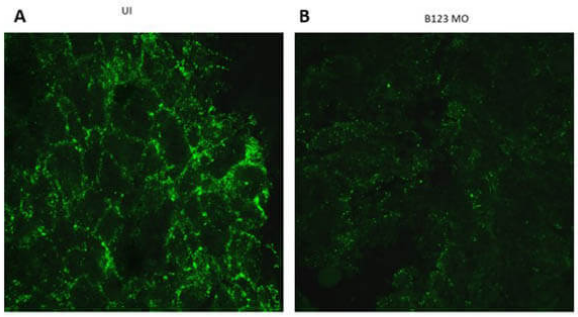
Immunofluorescence Microscopy
(B) MOs (4 ng) and NTB constructs (50 pg) were injected into one D1.1 blastomere at the 16-cell stage as indicated. Brains were dissected at stage 35 and immunostaining performed using anti-ephrinB and phospho-histone H3 antibodies. In the NTB panels, overexpressed ephrinB1-HA was stained with anti-HA-Alexa Fluor 488, whereas membrane GFP was stained with anti-GFP-Alexa Fluor 488. Yellow dotted lines indicate telencephalon borders. Fig 7. PMID: 33462110



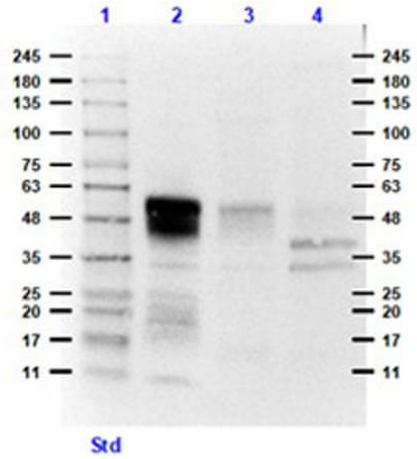
Immunofluorescence Microscopy
(B) MOs (4 ng) and RNAs (200 pg) were injected into one D1 blastomere at the eight-cell stage as indicated. Brains were dissected at stage 45 and immunostaining performed using anti-ephrinB and phospho-histone H3 antibodies. Insets show red fluorescent protein (RFP), indicating injected side, and DAPI. Fig 6. PMID: 33462110



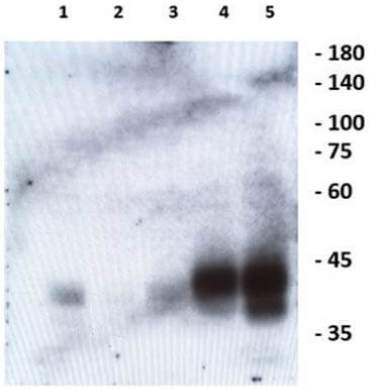
Immunofluorescence Microscopy
(B) Brains were dissected from Rab11fip5 KO tadpoles at stage 45. Immunostaining was performed with anti-ephrinB antibodies. Insets show red fluorescent protein (RFP), indicating injected side. Fig 4. PMID: 33462110



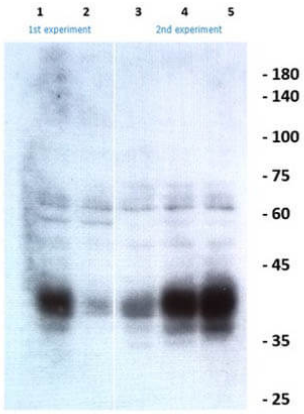
Immunofluorescence Microscopy
Immunofluorescence of Rabbit Anti-Ephrin B1-B3 Antibody. (A). Neural Plate of Xenopus laevis stage 17. (B). Knockdown neural plate of Xenopus laevis stage 17. Primary Antibody: Anti-Ephrin B1-3 at 1:5000 overnight at 2-8°C. Secondary Antibody: Anti-Rabbit IgG Fluorescent Secondary. Fixative: 4% Paraformaldehyde in PBS. Permeabilization: 0.05% Triton-X100 in PBS. Nuclear Counterstain: None. Magnification: 63X objective, Zeiss LSM710. Results: Ephrin B1 B2 B3 triple knockdown with a mixture of all three morpholinos abolished most of the signal which can be seen at expected plasma membrane and vesicle subcellular locations.



Western Blot
Western Blot of Rabbit Anti-Ephrin B1-B3 Antibody. Lane 1: Opal PreStain Molecular Weight Marker (p/n MB-210-0500). Lane 2: EPHRIN B1 overexpressing HEK293T Lysate (10µg) [+]. Lane 3: HEK293T Lysate (10µg) [-]. Lane 4: Mouse Kidney Lysate (p/n W10-000-T017) (35µg) [+]. Primary Antibody: Anti-Ephrin B1-B3 at 1:1000 overnight at 2-8°C. Secondary Antibody: Goat anti-Rabbit IgG MX10 HRP (p/n 611-103-122) at 1:70,000 for 30 min at RT. Block: BlockOut Buffer (p/n MB-073). Predicted MW: ~38kDa endogenous, ~37-45kDa due to glycosylation. Observed: ~46-52kDa overexpressed, ~34, ~40kDa endogenous.



Western Blot
Western Blot of Rabbit Anti-Ephrin B1-B3 Antibody. Lane 1: Uninjected Xenopus laevis heterogenous embryo control (20µl). Lane 2: Morpholino Knockdown of Ephrin B1/2 heterogenous embryo Lot#1 (20µl). Lane 3: Morpholino Knockdown of Ephrin B1/2 heterogenous embryo Lot#2 (20µl). Lane 4: Ephrin B1-HA overexpression heterogenous embryo lysate (20µl). Lane 5: Ephrin B2-HA overexpression heterogenous embryo lysate (20µl). Primary Antibody: Anti-Ephrin B1-B3 at 1:2000 overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:5000 for 60 mins at RT. Block: 5% skim milk/TBST/0.1% Tween for 30 mins at RT. Predicted MW: ~38kDa. Observed MW: ~37-45kDa (due to glycosylation). Exposure: 10 sec. Results: Ephrin B1,2, double knockdown with a mixture of both morpholinos oligomers abolished most of the WB signal at expected size which was double checked by over-expression.



Western Blot
Western Blot of Rabbit Anti-Ephrin B1-B3 Antibody. Lane 1: 1st experiment: Uninjected heterogenous embryo [+]. Lane 2: 1st experiment: Ephrin B1,2 MO (2 morpholino oligomers). Lane 3: 2nd experiment: Ephrin B1,2,3 MO (3 morpholino oligomers). Lane 4: 2nd experiment: Ephrin B3 MO (1 morpholino oligomer). Lane 5: 2nd experiment: Uninjected heterogenous embryo [+]. Primary Antibody: Anti-Ephrin B1-B3 at 1:2000 overnight at 2-8°C. Secondary Antibody: Goat anti-Rabbit IgG HRP (p/n 611-103-122) 1:5000 for 60 min at RT. Block: 5% skim milk/TBST/0.1% Tween. Exposure: 10 sec. Predicted MW: ~38kDa. Observed: ~37-45kDa. Results: Ephrin B1,2,3 triple knockdown with a mixture of all three morpholinos abolished significant amount of the WB signal at expected size. The comparison of lane 3 and 4 suggests not much endogenous Ephrin B3 expression exist in this stage embryos.

References

- Yoon J et al. Wnt4 and ephrinB2 instruct apical constriction via Dishevelled and non-canonical signaling. *Nat Commun.* (2023)
- Yoon J et al. Rab11fip5 regulates telencephalon development via ephrinB1 recycling. *Development.* (2021)

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