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Datasheet for 600-401-P16S

Anti-mCherry Antibody Pre-Adsorbed

Overview

Description:	Anti-mCherry (RABBIT) Antibody (Min X Hu Ms & Rt Serum Proteins) - 600-401-P16S
Item No.:	600-401-P16S
Size:	25 µL
Applications:	ELISA, WB, IF, IHC, IP, Multiplex
Reactivity:	RFP, mCherry, rRFP
Host Species:	Rabbit

Product Details

Background:	mCherry antibody is ideal for western blotting. Fluorescent proteins such as Discosoma Red Fluorescent Protein (and its variants), and GFP are widely used in research practice. Both commonly serve as a markers for gene expression and protein localization. DsRed was isolated from sea anemone Discosoma sp. mushroom and GFP is originated from Aequorea victoria jellyfish. As DsRed and GFP share only 19% identity, therefore, in general, anti-GFP antibodies do not recognize DsRed protein and vice versa. Structurally, Discosoma red fluorescent protein is similar to Aequorea green fluorescent protein in terms of its overall fold (a β-can) and chromophore-formation chemistry. However, Discosoma red fluorescent protein undergoes an additional steps in the chromophore maturation and obligates tetrameric structure. All mCherry antibodies have been pre-absorbed to eliminate any potential cross-reactivity to human, mouse and rat serum proteins. The antibodies are also confirmed for non-reactivity to GFP protein.
Synonyms:	rabbit anti-mCherry antibody, RFP, mCherry monomeric red fluorescent protein, Red Fluorescent Protein (RFP), rDsRed, Discosoma sp. Red Fluorescent Protein
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	DsRed
Reactivity:	RFP, mCherry, rRFP

Immunogen:	The immunogen is a mCherry mutant variant fusion protein of RFP corresponding to the full length amino acid sequence (234aa) derived from the mushroom polyp coral Discosoma.
Purity/Specificity:	mCherry was prepared from monospecific antiserum by immunoaffinity chromatography using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Expect reactivity against mCherry, RFP and its variants: tdTomato, mBanana, mOrange, mPlum, mOrange and mStrawberry. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum and purified and partially purified mCherry. No reaction was observed against Human, Mouse or Rat serum proteins. ELISA was used to confirm specificity at less than 0.1% of target signal.
Relevant Links:	• 600-401-P16 SDS

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IF, IHC, IP, Multiplex (Based on references)
Application Note:	Polyclonal anti-mCherry is designed to detect mCherry, RFP, and its variants. Anti-mCherry (Discosoma sp.) has been tested by ELISA and Western blot and is intended for use in immunological assays including ELISA, western blotting, immunofluorescence, and fluorescence activated cell sorting (FACS). Researchers should determine optimal titers for applications that are not stated. In addition, we performed conjugation of RFP antibodies to either fluorescent dyes, biotin or horseradish peroxidase to further facilitate RFP protein detection and quantification.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:150,000 - 1:250,000
IF:	1:200 - 1:2,000
IHC:	1:200 - 1:2,000
WB:	1:2,000 - 1:10,000

Formulation

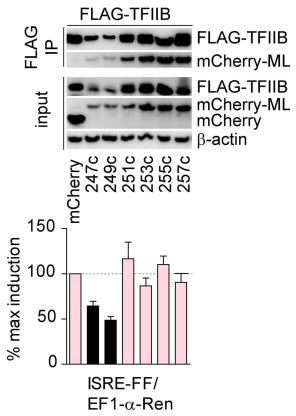
Physical State:	Liquid (sterile filtered)
Concentration:	1.04 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

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.

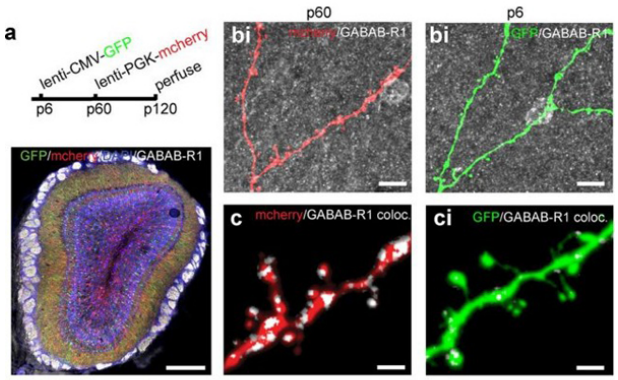
Images

C

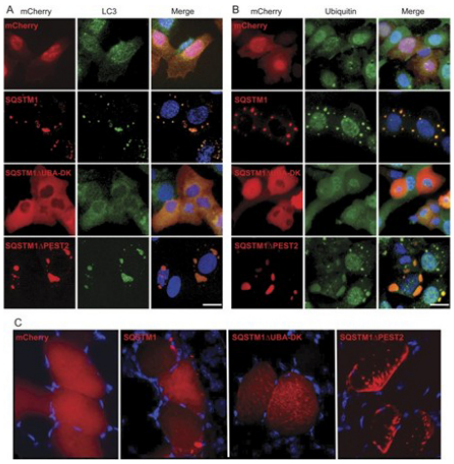


Western Blot

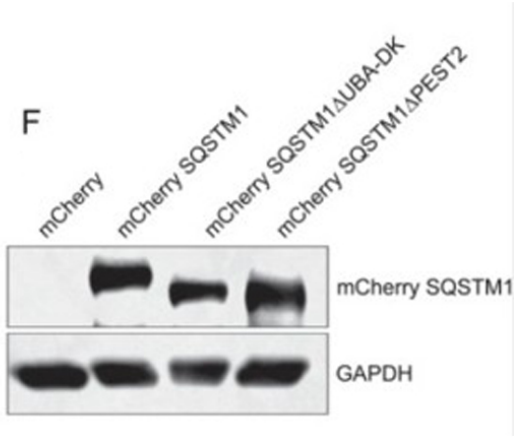
Mapping of a minimal ML sequence required for TFIIB inhibition.A) Schematic representation of ML fragments. B) Top panel: IP of FLAG-tagged TFIIB and GFP-fused ML fragments. Bottom panel: reporter assay in HEK293 cells, where Firefly luciferase under ISRE promoter was co-transfected with EF1-α-Renilla and GFP-ML fragments. C) Top-panel: IP of FLAG-tagged TFIIB and mCherry-fused ML fragments. Bottom panel: reporter assay in HEK293 cells, where Firefly luciferase under ISRE promoter was co-transfected with EF1-α-Renilla and mCherry-ML fragments. Western blots are representative of two experiments with similar results. Bar graphs show mean and SD from three technical replicates and are representative of two experiments with similar results. Figure provided by CiteAb. Source: PLoS Pathog, PMID: 29709033.



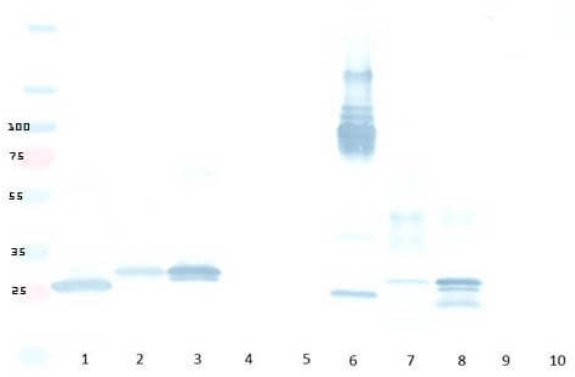
Immunofluorescence Microscopy
GABAB1(a,b)R immunofluorescent density varies with cell birth date. a, Viral injection schedule (top) and double-infected OB costained with antibody specific to GABAB1 (a,b)R (see Materials and Methods; bottom). Scale bar, 400 µm. b, Example apical dendrites of P60 (red, b) or P6 (green, bi). Scale bar, 10 µm. c, Magnification of maximal intensity projection of colocalized GABAB1(a,b)R immunoreactivity (white) of P60 (red, c) or P6 (green, ci) GCs. Scale bar, 2 µm. Fig 4. PMID: 24027267



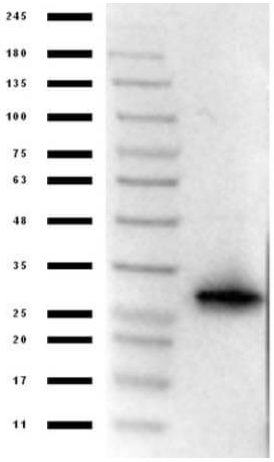
Immunofluorescence Microscopy
(A, B) U2OS cells were transiently transfected with plasmids expressing mCherry alone or mCherry fused to SQSTM1, SQSTM1ΔUBA-DK, or SQSTM1ΔPEST2 and immunostained with an antibody to (A) microtubule-associated protein 1A light chain-3 (LC3) or (B) ubiquitin. SQSTM1ΔUBA-DK is present diffusely throughout the cell and does not colocalize with LC3 or ubiquitin, whereas SQSTM1ΔPEST2 is present as large perinuclear inclusions that colocalize with both LC3 and ubiquitin. (C) Mouse tibialis anterior muscle was electroporated with plasmids expressing mCherry alone or mCherry fused to SQSTM1, SQSTM1ΔUBA-DK, or SQSTM1ΔPEST2, sectioned, and subjected to fluorescence microscopy. SQSTM1 is present both throughout the sarcoplasm and as larger inclusions. SQSTM1ΔUBA-DK does not form inclusions and is present on myofibrillar structures. In contrast, SQSTM1ΔPEST2 is present as large subsarcolemmal and sarcoplasmic inclusions. Fig 4. PMID: 26208961



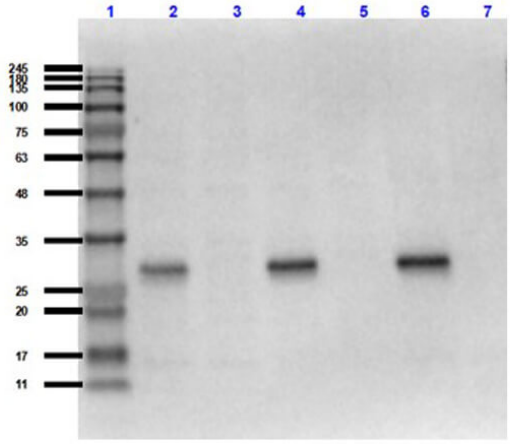
Western Blot
(F) Immunoblot for SQSTM1 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from lysates of U2OS cells transiently transfected with plasmids expressing mCherry alone or mCherry fused to SQSTM1, SQSTM1ΔUBA-DK, or SQSTM1ΔPEST2. Fig 3. PMID: 26208961



Western Blot
Western Blot of Rabbit anti-mCherry antibody. Lane 1: Rockland mCherry fusion protein (reduced). Lane 2: Control dsRed reduced. Lane 3: Control mCherry reduced. Lane 4: Control BFP reduced. Lane 5: Control eGFP reduced. Lane 6: Rockland mCherry fusion protein (non-reduced). Lane 7: Control dsRed non-reduced. Lane 8: Control mCherry non-reduced. Lane 9: Control BFP non-reduced. Lane 10: Control eGFP non-reduced. Total load ~200 nanograms per lane. Load: 200ng per lane. Primary Antibody: Anti-mCherry at 1:5000 for overnight at 4°C. Secondary antibody: HRP rabbit secondary antibody at 1:10,000 and TMBM-100. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 25.9 kDa, for mCherry and RFP, no reaction to BFP or GFP.



Western Blot
Western Blot of Rabbit Anti-mCherry Antibody. Lane 1: Opal Prestained Marker (p/n MB-210-0500). Lane 2: 50ng of RFP. Primary Antibody: rabbit anti-mCherry at 1µg/mL overnight at 4°C. Secondary Antibody: goat anti-Rabbit peroxidase (p/n 611-103-122) at 1:70000 for 30mins at RT. Block: BlockOut Universal Buffer (p/n MB-073). Expect band ~30 kDa.



Western Blot
Western Blot of Rabbit Anti-mCherry Antibody MX Hu Ms Rt. Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 2: RFP (p/n 000-001-379)/HeLa WCL (p/n W09-000-364) [0.02µg/10µg]. Lane 3: HeLa WCL (p/n W09-000-364) [10µg]. Lane 4: RFP (p/n 000-001-379)/NIH/3T3 WCL (p/n W10-000-358) [0.02µg/10µg]. Lane 5: NIH/3T3 WCL (p/n W10-000-358) [10µg]. Lane 6: RFP (p/n 000-001-379)/PC-12 WCL (p/n W12-001-GL9) [0.02µg/10µg]. Lane 7: PC-12 WCL (p/n W12-001-GL9) [10µg]. Primary Antibody: Anti-mCherry at 1:1000 overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:70,000 for 30mins at RT. Block: BlockOut Buffer (p/n MB-073). Predicted MW: ~27-30kDa.

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