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Datasheet for 600-401-B06S

c-Myb Antibody

Overview

Description:	Anti-c-Myb (RABBIT) Antibody - 600-401-B06S
Item No.:	600-401-B06S
Size:	25 µL
Applications:	ELISA, WB, IHC, Multiplex
Reactivity:	Mouse, Monkey
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) and is suitable for Cancer, Immunology and Nuclear Signaling research. The transcription factor c-Myb plays an important role in the development of all hematopoietic lineages during definitive hematopoiesis. Its critical role in hematopoiesis has been first demonstrated in experiments with targeted disruption of the c-Myb gene where deletion of both alleles of the c-Myb gene was embryonally lethal due to severe anemia. c-Myb is a short-lived sequence specific DNA-binding protein that regulates transcription of several important genes involved directly in cellular processes such as proliferation, differentiation, and apoptosis. Post-translational modifications such as phosphorylation, acetylation, ubiquitination, and conjugation of SUMO proteins are crucial for modulation of the transactivation activity of c-Myb.
Synonyms:	rabbit anti-c-Myb Antibody, Proto-oncogene c-Myb antibody, C myb antibody, MYB antibody, Transcriptional activator Myb, cmyb antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Myb
Reactivity:	Mouse, Monkey

Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a peptide corresponding to an internal portion of mouse c-Myb protein.
Purity/Specificity:	This product was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody reacts with endogenous c-Myb protein and also SUMOylated overexpressed c-Myb protein in transfected Cos7 cells. A BLAST analysis was used to suggest reactivity with c-Myb from mouse based on a 100% homology with the immunizing sequence. Expect reactivity with c-Myb from rat, pig, human, horse, platypus, opossum, orangutan, spider monkey, bonobo, gorilla, and chimpanzee; with a v-myb myeloblastosis viral oncogene homolog, all isoforms (also avian version) from human; and with a protooncogene c-myb, and a v-myb myeloblastosis viral oncogene homolog (avian), both from cattle, all based on a 93% homology with the immunizing sequence. Cross-reactivity with c-Myb from other sources has not been determined.
Relevant Links:	• NCBI - 110556654 • UniProtKB - P06876 • GeneID - 17863

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IHC, Multiplex (Based on references)
Application Note:	This affinity purified antibody has been tested for use in ELISA and western blotting using M1 cells which express endogenous wild-type c-Myb protein and in COS7 cells transfected with c-Myb. Specific conditions for reactivity and detection of c-Myb should be optimized by the end user. Expect a band approximately ~75 kDa in size corresponding to c-Myb by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:25,000
WB:	1:250 – 1:500

Formulation

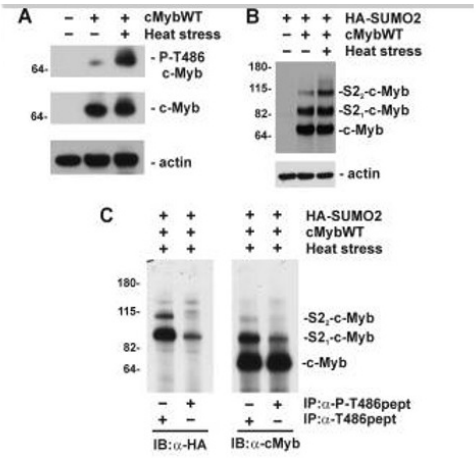
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

Stabilizer:	None

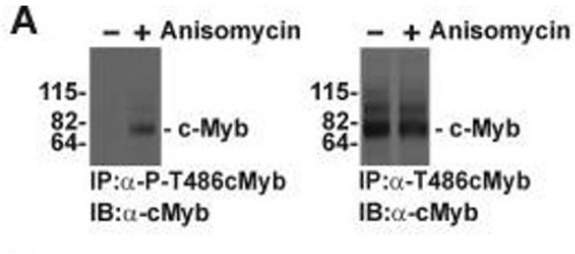
Shipping & Handling

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 three (3) months from date of receipt.

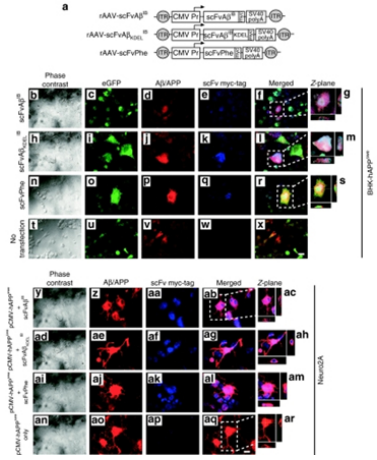
Images



Immunoprecipitation
Phosphorylation of Thr486 negatively regulates stress-induced SUMOylation of c-Myb.
A). COS7 cells were transfected with a construct encoding c-MybWT and treated with hyperthermia as indicated. Immunoblotting results are of total cell lysates analyzed with rabbit polyclonal antibody specific for phosphorylated Thr486 (P-cMybT486 p/n 600-401-C47, top panel), nonphosphorylated Thr486 in murine c-Myb (c-Myb p/n 600 -401-B06, middle panel), or anti-actin antibody (bottom panel).
B). cell lysates from COS7 cells transfected with construct encoding c-MybWT and HA-SUMO-2 and treated with hyperthermia were analyzed by immunoblotting with monoclonal anti-c-Myb (top panel), or anti-actin antibody (bottom panel). The mobility of unmodified (c-Myb) and SUMOylated forms (HAS21-cMyb and HAS22-cMyb) are marked.
C). immunoprecipitation of c-Myb using rabbit polyclonal antibody specific for phosphorylated (α-P-T486pept p/n 600-401-C47) or nonphosphorylated Thr486 (α-T486pept p/n 600-401-B06) were analyzed by immunoblotting with monoclonal anti-HA (α-HA, left panel) or anti-c-Myb antibody (α-cMyb, right panel).
Fig 3. PMID: 24257756

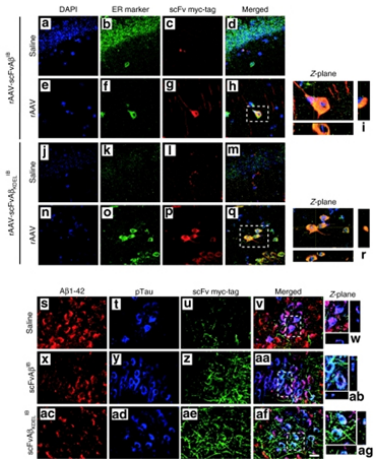


Immunoprecipitation
Regulation of endogenous c-Myb SUMOylation by p38 MAPKs in myeloid cells. A, M1 cells expressing endogenous c-Myb and SUMO-2/3 were treated with anisomycin for 20 min as indicated, and c-Myb was immunoprecipitated with rabbit polyclonal antibody specific for phosphorylated (IP:α-P-T486cMyb, p/n 600-401-C47 left panel) or nonphosphorylated Thr486 (IP:α-T486cMyb, p/n 600-401-B06 right panel). Immunoprecipitates were analyzed by immunoblotting with monoclonal anti-c-Myb antibody (IB:α-cMyb). Fig 5. PMID: 24257756

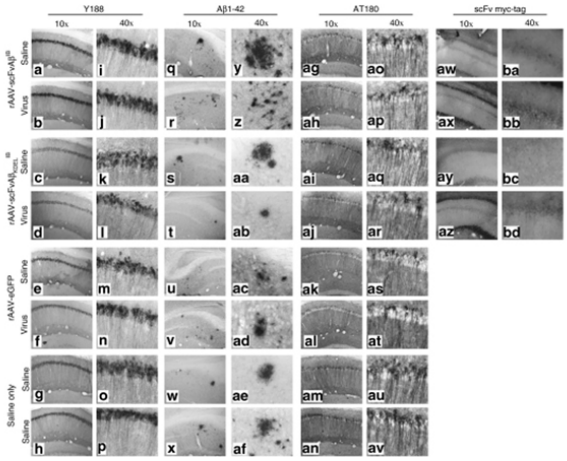


Immunofluorescence Microscopy
Colocalization of anti-Aβ42 intrabodies with hAPPswe/Aβ in vitro. (a) Two recombinant adeno-associated virus (rAAV) vectors were constructed: one expressing an Aβ42-specific intrabody sequence with a c-myc epitope tag at the C-terminus to facilitate immunocytochemical detection (rAAV-scFvAβIB), and a second expressing the same c-myc tagged intrabody but with an endoplasmic reticulum targeting signal (KDEL) inserted in-frame between the intrabody coding sequence and c-myc epitope (rAAV-scFvAβKDELIB). The individual transgenes were placed under the transcriptional control of the human cytomegalovirus (CMV) promoter. A polyadenylation signal from SV40 was included at the 3′ end of the transcription unit, which in total was flanked by AAV inverted terminal repeat sequences. A rAAV vector expressing a phenobarbital-specific scFv that had been described previously was used as a negative control for a subset of in vitro studies.45 The rAAV-scFvAβIB (b–g), rAAV-scFvAβKDELIB (h–m), and rAAV-scFvPhe (n–s) plasmids were transiently transfected into the baby hamster kidney (BHK)-human amyloid precursor protein Swedish mutant (hAPPswe) cells incubated in the presence of 2 μg/ml doxycycline (Dox), whereas nontransfected, Dox-treated BHK-hAPPswe cells were used as negative controls (t–x). Forty-eight hours post-transfection, coimmunocytochemistry was performed for eGFP (green; c,i,o,u), hAPP/Aβ (red; d,j,p,v), and the c-myc epitope tag (blue; e,k,q,w). Images were obtained by confocal fluorescence microscopy at ×40 original magnification. Co-registered green/red/blue staining is indicated in the merged images as white (f,g,l,m,r,s,x). Co-registered green/red staining is indicated in the merged images as yellow.

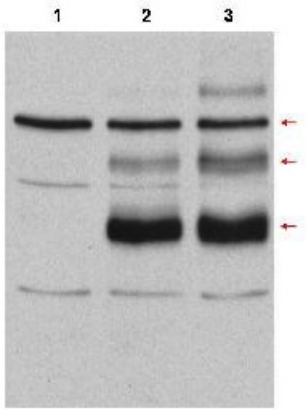
Panels g,m, and s represent Z-plane images of regions in f,l, and r demarcated with a white dotted box. The rAAV-scFvAβIB (y–ac), rAAV-scFvAβKDELIB (ad–ah), and rAAV-scFvPhe (ai–am) plasmids were also transiently co-transfected with a plasmid expressing hAPPswe into Neuro2A cells with, while Neuro2A cells transfected with only pCMV-hAPPswe were used as controls (an–ar). Forty-eight hours post-transfection, coimmunocytochemistry was performed for hAPP/Aβ (red; z,ae,aj,ao) and the c-myc epitope tag (blue; aa,af,ak,ap). Images were obtained by confocal fluorescence microscopy at ×40 original magnification. Co-registered red/blue staining is indicated in the merged images as pink (ab,ac,ag,ah,al,am).
Panels ac,ah,am, and ar represent Z-plane images of regions in f,l, and r. Bars in x and aq = 10 µm. scFv, single-chain variable fragment; Aβ, amyloid-β; IB, intrabody. Fig 2.
PMID: 19638957



Immunofluorescence Microscopy
AAV vector–mediated expression of the Aβ-specific intrabodies in 3xTg-AD mice reveals the intrabodies alter general patterns of cell-associated Aβ42. Two month-old 3xTg-AD mice were unilaterally injected in the CA1 region of the hippocampus with saline (a–d and j–m), recombinant adeno-associated virus (rAAV)-scFvAβIB (e–i) and rAAV-scFvAβKDELIB (n–r). Nine months postinjection animals were killed and brain sections were processed for coimmunocytochemistry with the following: 4',6-diamidino-2-phenylindole (blue; a,e,j,n), endoplasmic reticulum-specific antibody (green; b,f,k,o) and c-myc epitope tag-specific antibody (red; c,g,l,p). Co-registered staining is indicated in the merged images as yellow (d,h,m,q). Panels i and r represent digitally magnified images, which illustrate a cross section through the Z-plane obtained with confocal microscopy. All images were obtained at ×100 original magnification. Additional brain sections from these rAAV vector-injected 3xTg-AD mice were processed for fluorescence coimmunocytochemistry with the following: Aβ42-specific antibody (red; s,x,ac), phospho-Tau (AT180)-specific antibody (blue; t,y,ad) and c-myc epitope tag-specific antibody (green; u,z,ae). Co-registered staining for all markers is indicated in the merged images as white or for Aβ42 and phospho-Tau as purple (v,w,aa,ab,af,ag). Panels w,ab, and ag represent digitally magnified images, which illustrate a cross section through the Z-plane obtained with confocal microscopy. All images were obtained at ×100 original magnification. scFv, single-chain variable fragment; Aβ, amyloid-β; IB, intrabody. Fig 4.
PMID: 19638957



Immunohistochemistry
Qualitative effects of chronic anti-Aβ42 intrabody expression on AD-related pathological hallmarks. Two month-old 3xTg-Alzheimer's disease mice were unilaterally injected in the CA1 region of the hippocampus with saline, recombinant adeno-associated virus (rAAV)-enhanced green fluorescent protein, rAAV-scFvAβIB or rAAV-scFvAβKDELIB. Nine months postinjection animals were killed and 30-µm brain sections were processed for immunohistochemical analyses of hAPP transgene expression (Y188 antibody; a–p), extracellular Aβ 1–42 deposition (anti-Aβ42 antibody; q–af), hyperphosphorylated Tau (AT180 antibody; ag–av), and c-myc epitope tag-specific antibody (aw–bd). Of note, the images obtained for the c-myc epitope tag immunohistochemical analysis were obtained originally in color by fluorescence microscopy, converted to black-and-white, and subsequently inverted to provide comparable views to the other sections captured using conventional light microscopy following 3,3'-diaminobenzidine immunohistochemistry. Representative images of the infused hippocampus are displayed at ×10 (a–h,q–x,ag–an,aw–az) and ×40 original magnification (i–p,y–af,ao–av,ba–bd). scFv, single-chain variable fragment; Aβ, amyloid-β; IB, intrabody. Fig 5. PMID: 19638957



Western Blot
Western Blot of Rabbit anti-C-Myb antibody. Lane 1: Cos7 cell lysates vector. Lane 2: Cos7 cell lysates transfected with c-myb, no treatment. Lane 3: Cos7 cell lysates transfected with c-Myb and heat stress-treated (incubation at 42°C for 30 minutes) to induce c-Myb modification. Load: 35 µg per lane. Primary antibody: cMyb antibody at 1:500 for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 10% BLOTTO overnight at 4°C. Predicted/Observed size: Lane 3 shows detection of bands at 75, 98, and 125kDa corresponding to overexpressed c-Myb (arrows): wild-type, with one attached SUMO molecule, and with two attached SUMO molecules, respectively.

References

- Sudol K et al. Generating differentially targeted amyloid-beta specific intrabodies as a passive vaccination strategy for Alzheimer's disease. *Molecular Therapy : the Journal of the American Society of Gene Therapy* (2009)

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