Order online now!

www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for 600-401-C47S

C-Myb phosphoT486 Antibody

Overview

Description:	Anti-c-Myb pT486 (RABBIT) Antibody - 600-401-C47S
Item No.:	600-401-C47S
Size:	25 µL
Applications:	ELISA, WB, IP
Reactivity:	Mouse
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 pT486 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

PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic phospho-peptide corresponding to an internal region of mouse c-Myb protein surrounding amino acid residue 486.
Purity/Specificity:	This affinity-purified antibody is directed against the phosphorylated form of mouse c-Myb protein at the pT486 residue. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against mouse c-Myb pT486 protein and the antibody is specific for the phosphorylated form of the protein. This antibody reacts with endogenous c-Myb protein and also SUMOylated overexpressed c-Myb protein in transfected Cos7 cells. Reactivity with non- phosphorylated mouse c-Myb is minimal by ELISA and western blot. 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:	IP (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 phosphorylated c-Myb should be optimized by the end user. Expect a band approximately ~75 kDa in size corresponding to phosphorylated 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:	1:5,000-1:16,000
WB:	1:2,500

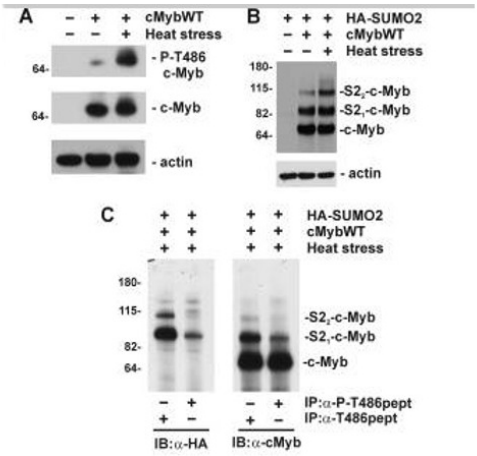
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.7 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

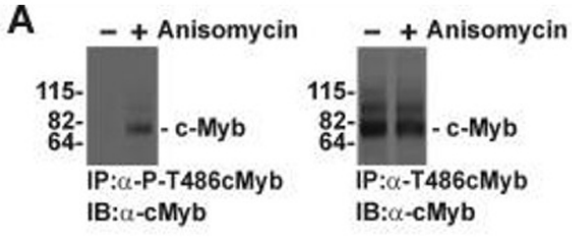
Shipping & Handling

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.

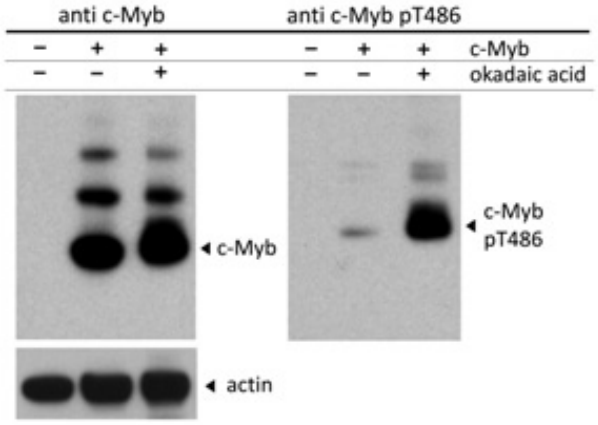
Images



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



Western Blot
Western Blot of Rabbit c-Myb pT486 antibody. Lane 1: Cos7 cells were either null for c-Myb. Lane 2: Cos7 cell lysates transfected with c-myb, no treatment. Lane 3: Cos7 cell lysates transfected with c-Myb and treated with okadaic acid to include phosphorylation of c-Myb. Load: 35 µg per lane. Primary antibody: The left blot was probed with anti-c-Myb antibody, the right blot was probed with anti-c-Myb pT486 antibody at 1:2500 for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: PBS-Tween (0.05%) overnight at 4°C. Predicted/Observed size: 75, 98, and 125kDa corresponding to overexpressed c-Myb (arrow): wild-type, with one attached SUMO molecule, and with two attached SUMO molecules, respectively.

References

- Bies J, Sramko M, Wolff L. Stress-induced phosphorylation of Thr486 in c-Myb by p38 mitogen-activated protein kinases attenuates conjugation of SUMO-2/3. *J Biol Chem.* (2013)

Disclaimer