ENCODE DCC Antibody Validation Document

Date of Submission: Aug 30, 2011

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Lab: Myers

Antibody Name: MYBL2_(SC-81192)

Target: MYBL2

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-81192

Lot Number: B1611

Antibody Description: Mouse monoclonal IgG raised against a recombinant protein corresponding to an internal region of B-Myb of human origin.

Target Description: The protein encoded by this gene, a member of the MYB family of transcription factor genes, is a nuclear protein involved in cell cycle progression.

Entrez gene ID 4605; 79 kDa

Species Target: Human

Species Host: Mouse

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Monoclonal

Vendor URL: http://www.scbt.com/datasheet-81192-b-

Reference (PI/ Publication Information):

Please complete the following for antibodies to histone modifications:

if your specifications are not listed in the drop-down box, please write-in the appropriate information

Histone Name:

AA modified:

AA Position:

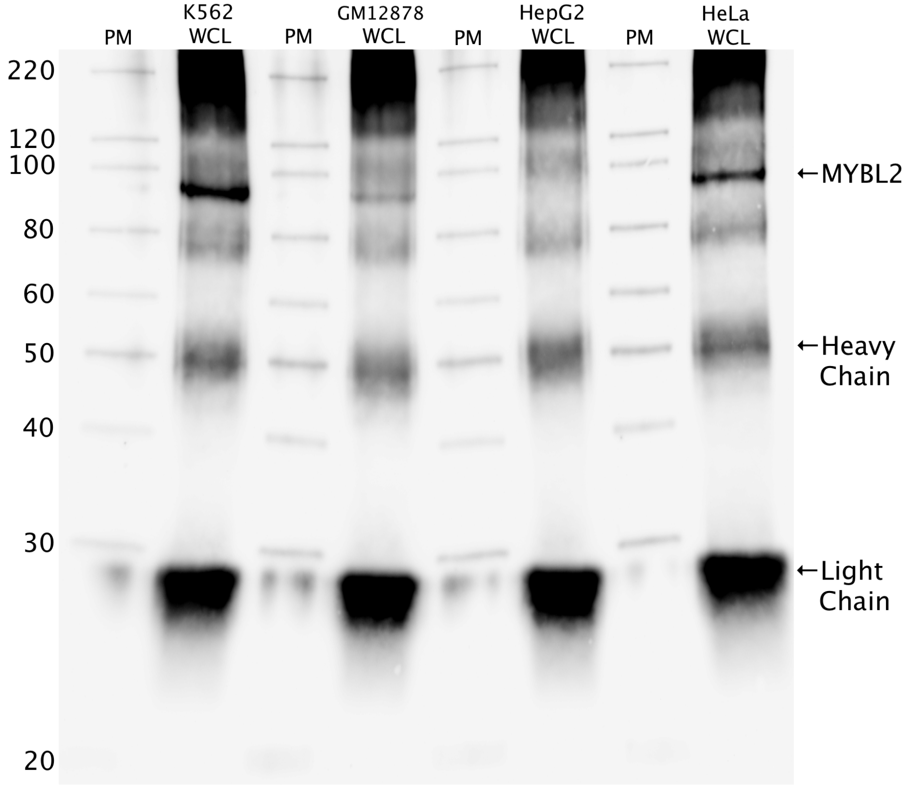
Modification:

Validation #1
Analysis

Western blot protocol:
Whole cell lysate was immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. After separation, the samples were transferred to a nitrocellulose membrane with an Invitrogen iBlot system. Blotting with primary (same as that used for IP) and secondary HRP-conjugated antibodies was performed on an Invitrogen BenchPro 4100 system. Visualization was achieved using SuperSignal West Femto solution (Thermo Scientific).

Results: Band of expected size visualized, representing strongest signal in the lane.

Figure legend: IP-western with sc-81192 in whole cell lysate (WCL) of K562, GM12878, HepG2 and HeLa; PM=protein marker. MYBL2 band is indicated.



Insert Validation Image (click here)

Validation #2 Analysis

IP followed by mass spectrometry:
Briefly, K562 whole cell lysate was immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. Gel was stained with Coomassie Blue in order to visualize marker bands. Gel fragments corresponding to the bands indicated above in the western blot image were excised and sent to the University of Alabama at Birmingham Cancer Center Mass Spectrometry/Proteomics Shared Facility. There the samples were run on an LTQ XL Linear Ion Trap Mass Spectrometer by LC-ESI-MS/MS. Peptides were identified using SEQUEST tandem mass spectra analysis, with probability based matching at $p < 0.05$.

As per ENCODE data standards, all SEQUEST results are attached (ENCODE_HAIB_MYBL2_sc81192_08252011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 3a in the ~90 kDa band.

Insert Validation Image (Click here)

Submit by Email

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14 entries (4 single hits) retrieved from
/home/TPP/tpp/20110617_Bowling/int-gb_20110617_LTQ_Bowling_MYBL2-sequest.prot.xml


* corresponds to peptide is_nondegenerate_evidence flag


1       UniRef100_P54886 UniRef100_P54886-2 1.0000
        confidence: 1. max coverage num unique p tot indep spec share of spectrum id's: 1.44%

        >Gamma-glutamyl phosphate reductase n=2 Tax=Homo sapiens RepID=P5CS_HUMAN                          Length: 795aa
        >Isoform Short of Delta-1-pyrroline-5-carboxylate synthase n=1 Tax=Homo sapiens RepID=P54886-2


2a      UniRef100_A4D2A2 UniRef100_B2RBA6 UniRef100_B4DDF5 UniRef100_C6EMX8 UniRef100_P33993 1.0000
        confidence: 1. max coverage num unique p tot indep spec share of spectrum id's: 0.71%

        >MCM7 minichromosome maintenance deficient 7 (S. cerevisiae) n=2 Tax=Homo sapiens RepID=A4D2A2_HUMAN   Length: 543aa

        >cDNA, FLJ95407, highly similar to Homo sapiens MCM7 minichromosome maintenance deficient 7 (S. cerevisiae) (MCM7), mRNA n=1

                  Tax=Homo sapiens RepID=B2RBA6_HUMAN

        >cDNA FLJ55929, highly similar to DNA replication licensing factor MCM7 n=1 Tax=Homo sapiens RepID=B4DDF5_HUMAN
        >HsMcm7 n=1 Tax=Homo sapiens RepID=C6EMX8_HUMAN
        >DNA replication licensing factor MCM7 n=1 Tax=Homo sapiens RepID=MCM7_HUMAN


3a      UniRef100_B7Z8D9 UniRef100_P10244 UniRef100_UPI00015DF89E 1.0000
        confidence:  max coverag num unique  tot indep spe share of spectrum id's: 1.79%

        >cDNA FLJ57697, highly similar to Myb-related protein B n=1 Tax=Homo sapiens RepID=B7Z8D9_HUMA Length: 676aa
        >Myb-related protein B n=1 Tax=Homo sapiens RepID=MYBB_HUMAN
        >Myb-related protein B (B-Myb). n=1 Tax=Homo sapiens RepID=UPI00015DF89E


4a      UniRef100_C5IWV5 UniRef100_P00761 1.0000
        confidence: 1. max coverage num unique p tot indep spec share of spect subsumed entries: 3

        >Trypsinogen n=1 Tax=Sus scrofa RepID=C5IWV5_PIG                                                    Length: 246aa
        >Trypsin n=1 Tax=Sus scrofa RepID=TRYP_PIG


5a      UniRef100_P02769 1.0000
        confidence: 1. coverage: 9.4 num unique p tot indep spec share of spect subsumed entries: 5

        >Serum albumin n=1 Tax=Bos taurus RepID=ALBU_BOVIN                                                  Length: 607aa


6a      UniRef100_P04264 1.0000
        confidence: 1. coverage: 29. num unique p tot indep spec share of spectrum id's: 13.20%

        >Keratin, type II cytoskeletal 1 n=1 Tax=Homo sapiens RepID=K2C1_HUMAN                              Length: 644aa


6b      UniRef100_P35908 1.0000
        confidence: 1. coverage: 22. num unique p tot indep spec share of spect subsumed entries: 2

        >Keratin, type II cytoskeletal 2 epidermal n=1 Tax=Homo sapiens RepID=K22E_HUMAN                    Length: 639aa


6c      UniRef100_P13647 1.0000
        confidence: 1. coverage: 7.8 num unique p tot indep spec share of spect subsumed entries: 3

        >Keratin, type II cytoskeletal 5 n=1 Tax=Homo sapiens RepID=K2C5_HUMAN                              Length: 590aa


7a      UniRef100_P08238 1.0000
        confidence: 1. coverage: 33. num unique p tot indep spec share of spect subsumed entries: 12

        >Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=HS90B_HUMAN                              Length: 724aa


7b      UniRef100_P14625 UniRef100_Q5CAQ5 1.0000
        confidence: 1. max coverage num unique p tot indep spec share of spect subsumed entries: 1

        >Endoplasmin n=1 Tax=Homo sapiens RepID=ENPL_HUMAN                                                  Length: 803aa
        >Tumor rejection antigen (Gp96) 1 n=1 Tax=Homo sapiens RepID=Q5CAQ5_HUMAN
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8a     UniRef100_P13639 1.0000
       confidence: 1. coverage: 7.6 num unique p tot indep spec share of spect subsumed entries: 5

       >Elongation factor 2 n=2 Tax=Hominidae RepID=EF2_HUMAN                                        Length: 858aa


9a     UniRef100_P13645 UniRef100_UPI00017BCE7F 1.0000
       confidence: 1. max coverage num unique p tot indep spec share of spect subsumed entries: 1

       >Keratin, type I cytoskeletal 10 n=1 Tax=Homo sapiens RepID=K1C10_HUMAN                       Length: 584aa
       >keratin, type I cytoskeletal 10 n=1 Tax=Homo sapiens RepID=UPI00017BCE7F


9b     UniRef100_P35527 1.0000
       confidence: 1. coverage: 17. num unique p tot indep spec share of spectrum id's: 5.29%

       >Keratin, type I cytoskeletal 9 n=1 Tax=Homo sapiens RepID=K1C9_HUMAN                         Length: 623aa


10a    UniRef100_Q4W4Y1 UniRef100_Q6NUS1 UniRef100_Q8WUM4 UniRef100_UPI00004121D3 1.0000
       confidence: 1. max coverage num unique p tot indep spec share of spect subsumed entries: 4

       >Dopamine receptor interacting protein 4 n=1 Tax=Homo sapiens RepID=Q4W4Y1_HUMAN              Length: 868aa
       >PDCD6IP protein n=1 Tax=Homo sapiens RepID=Q6NUS1_HUMAN
       >Programmed cell death 6-interacting protein n=1 Tax=Homo sapiens RepID=PDC6I_HUMAN
       >programmed cell death 6-interacting protein isoform 2 n=1 Tax=Homo sapiens RepID=UPI00004121D3


12a    UniRef100_UPI00017BDB3E UniRef100_UPI00017BDB43 1.0000
       confidence: 1. max coverage num unique p tot indep spec share of spect subsumed entries: 1
       >FabOX108 Heavy Chain Fragment n=1 Tax=Homo sapiens RepID=UPI00017BDB3E                      Length: 229aa
       >FabOX117 Heavy Chain Fragment n=1 Tax=Homo sapiens RepID=UPI00017BDB43


11a    UniRef100_UPI0000111654 UniRef100_UPI00017BDB3D UniRef100_UPI00017BDB42 0.9999
       confidence: 1. max coverage num unique p tot indep spec share of spectrum id's: 0.93%
       >MONOCLONAL ANTIBODY MAK33 n=1 Tax=Homo sapiens RepID=UPI0000111654                          Length: 213aa
       >FabOX108 Light Chain Fragment n=1 Tax=Homo sapiens RepID=UPI00017BDB3D
       >FabOX117 Light Chain Fragment n=1 Tax=Homo sapiens RepID=UPI00017BDB42


9c     UniRef100_P02533 0.9998
       confidence: 1. coverage: 6.6 num unique p tot indep spec share of spect subsumed entries: 3
       >Keratin, type I cytoskeletal 14 n=1 Tax=Homo sapiens RepID=K1C14_HUMAN                       Length: 472aa


4b     UniRef100_P06872 0.9997
       confidence: 1. coverage: 6.9 num unique p tot indep spec share of spectrum id's: 1.24%
       >Anionic trypsin n=2 Tax=Canis lupus familiaris RepID=TRY2_CANFA                              Length: 247aa


13     UniRef100_A5JHP3 UniRef100_P81605 0.9756
       confidence: 0. max coverage num unique p tot indep spec share of spectrum id's: 0.72%
       >Dermcidin isoform 2 n=1 Tax=Homo sapiens RepID=A5JHP3_HUMAN                                  Length: 121aa
       >DCD-1 n=3 Tax=Euarchontoglires RepID=DCD_HUMAN


14     UniRef100_B4DRF6 UniRef100_Q14566 UniRef100_Q4R6V0 0.9523
       confidence: 0. max coverage num unique p tot indep spec share of spectrum id's: 0.24%
       >cDNA FLJ51591, highly similar to DNA replication licensing factor MCM6 (Fragment) n=1 Tax=Homo sapiens RepID Length: 440aa
       >DNA replication licensing factor MCM6 n=2 Tax=Homo sapiens RepID=MCM6_HUMAN
       >Testis cDNA, clone: QtsA-17068, similar to human MCM6 minichromosome maintenance deficient 6 (MIS5homolog, S. pombe) (S. cerevisiae)
                  (MCM6), n=1 Tax=Macaca fascicularis RepID=Q4R6V0_MACFA

6d     UniRef100_Q0IIN1 UniRef100_UPI00001D797A 0.9518
       confidence: 1. max coverage num unique p tot indep spec share of spectrum id's: 0.90%
       >Keratin 77 n=1 Tax=Homo sapiens RepID=Q0IIN1_HUMAN                                           Length: 578aa
       >keratin, type II cytoskeletal 1b n=1 Tax=Homo sapiens RepID=UPI00001D797A
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