ENCODE DCC Antibody Validation Document

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Antibody Name: CEBPB_(SC-150)

Target: CEBPB

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-150

Lot Number: I1010

Antibody Description: Rabbit polyclonal IgG with epitope mapping at the C-terminus of C/EBP beta of rat origin.

Target Description: CEBPB CCAAT/enhancer binding protein (C/EBP), beta; 48 kD.

Entrez gene ID: 1051

Species Target: Human

Species Host: Rabbit

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method: ▼

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-150-c-ebp-

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

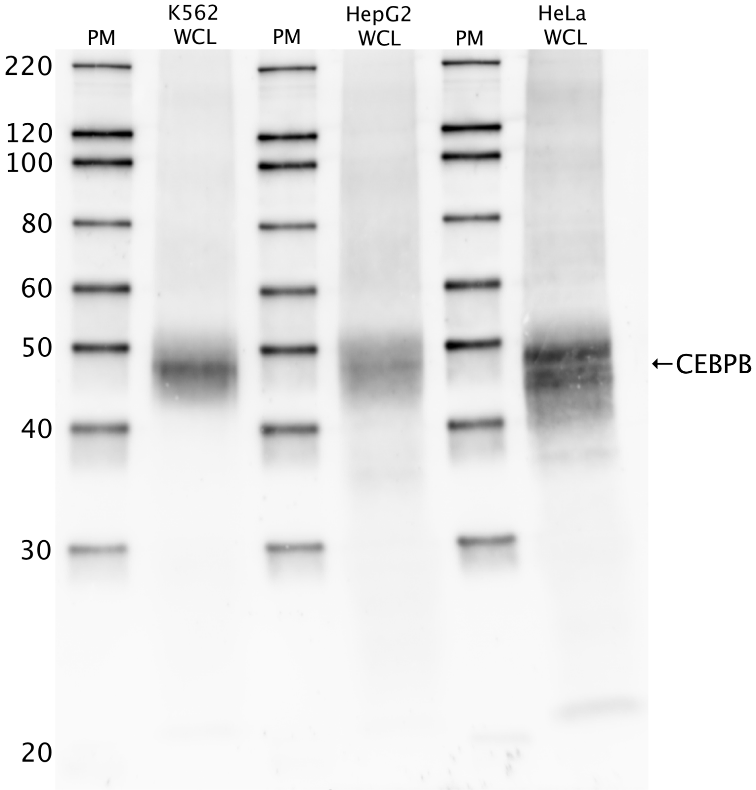
Histone Name ▼ AA modified ▼ AA Position ▼ Modification ▼

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. Band was tested by IP-mass spec.

Figure legend: IP-western with sc-150 in whole cell lysate (WCL) of K562, HepG2 and HeLa cells; PM=protein marker. CEBPB band is indicated.



←Molecular Weight of CEBPB: ~48kDa

Insert Validation Image (click here)

Validation #2 Analysis	IP followed by mass spectrometry: Briefly, K562 whole cell lysates were 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_CEBPB_(SC-150)_07192011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 19a in the ~48 kD band.

Insert Validation Image (Click here)

Submit by Email

34 entries (13 single hits) retrieved from
/home/TPP/tpp/20110617_Bowling/int-gb_20110617_LTQ_Bowling_CEBPB-sequest.prot.xml

* corresponds to peptide is_nondegenerate_evidence flag

1 UniRef100_B4DP56 UniRef100_P12277 UniRef100_UPI0001AE69A0 **1.0000**

confidence: 1.00	max coverage: 7.8%	num unique peps: 2	tot indep spectra: 5	share of spectrum id's: 1.16%

>cDNA FLJ52237, highly similar to Creatine kinase B-type (EC 2.7.3.2) n=1 Tax=Homo sapiens RepID=B4DP56_HUMAN Length: 346aa
>Creatine kinase B-type n=1 Tax=Homo sapiens RepID=KCRB_HUMAN
>UPI0001AE69A0 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE69A0

2 UniRef100_P35527 1.0000

confidence: 1.00	coverage: 21.5%	num unique peps: 10	tot indep spectra: 23	share of spectrum id's: 5.32%

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

3 UniRef100_P49411 1.0000

confidence: 1.00	coverage: 15.5%	num unique peps: 6	tot indep spectra: 14	share of spectrum id's: 3.26%

>Elongation factor Tu, mitochondrial n=1 Tax=Homo sapiens RepID=EFTU_HUMAN Length: 452aa

4 UniRef100_Q9N0W4 UniRef100_Q9N0W6 **1.0000**

confidence: 1.00	max coverage: 11.3%	num unique peps: 2	tot indep spectra: 6	share of spectrum id's: 1.40%

>Anti-human A33 heavy chain domain (Fragment) n=1 Tax=Oryctolagus cuniculus RepID=Q9N0W4_RABIT Length: 124aa
>Anti-human A33 heavy chain domain (Fragment) n=1 Tax=Oryctolagus cuniculus RepID=Q9N0W6_RABIT

10a UniRef100_B2RA03 UniRef100_P05783 **1.0000**

confidence: 1.00	max coverage: 19.8%	num unique peps: 8	tot indep spectra: 16	share of spectrum id's: 3.49%

>cDNA, FLJ94640, highly similar to Homo sapiens keratin 18 (KRT18), mRNA n=1 Tax=Homo sapiens RepID=B2RA03_HUMAN Length: 430aa
>Keratin, type I cytoskeletal 18 n=1 Tax=Homo sapiens RepID=K1C18_HUMAN

10b UniRef100_P13645 UniRef100_UPI00017BCE7F **1.0000**

confidence: 1.00	max coverage: 4.1%	num unique peps: 2	tot indep spectra: 3	share of spectrum id's: 0.86%

>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

11a UniRef100_B3KM39 UniRef100_Q9H0C8 **1.0000**

confidence: 1.00	max coverage: 33.2%	num unique peps: 18	tot indep spectra: 43	share of spectrum id's: 9.76%

>cDNA FLJ10181 fis, clone HEMBA1004227, highly similar to Homo sapiens integrin-linked kinase-associated serine/threonine phosphatase 2C (ILKAP), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=B3KM39_HUMAN Length: 392aa
>Integrin-linked kinase-associated serine/threonine phosphatase 2C n=1 Tax=Homo sapiens RepID=ILKAP_HUMAN

12a UniRef100_B4DTG2 UniRef100_P26641 **1.0000**

confidence: 1.00	max coverage: 5.3%	num unique peps: 2	tot indep spectra: 5	share of spectrum id's: 1.16%	subsumed entries: 1

>cDNA FLJ56389, highly similar to Elongation factor 1-gamma n=1 Tax=Homo sapiens RepID=B4DTG2_HUMAN Length: 487aa
>Elongation factor 1-gamma n=2 Tax=Homo sapiens RepID=EF1G_HUMAN

13a UniRef100_C5IWV5 UniRef100_P00761 **1.0000**

confidence: 1.00	max coverage: 24.7%	num unique peps: 24	tot indep spectra: 58	share of spectrum id's: 8.88%	subsumed entries: 2

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

14a UniRef100_O14929 UniRef100_Q6P594 **1.0000**

confidence: 1.00	max coverage: 7.8%	num unique peps: 2	tot indep spectra: 2	share of spectrum id's: 0.46%

>Histone acetyltransferase type B catalytic subunit n=2 Tax=Homininae RepID=HAT1_HUMAN Length: 419aa
>Histone acetyltransferase 1 n=1 Tax=Homo sapiens RepID=Q6P594_HUMAN

15a UniRef100_P02769 UniRef100_UPI000179EC85 **1.0000**

confidence:	max coverage:	num unique	tot indep	share of spectrum	subsumed
1.00	9.2%	peps: 6	spectra: 14	id's: 3.25%	entries: 3

>Serum albumin n=1 Tax=Bos taurus RepID=ALBU_BOVIN Length: 607aa
>Serum albumin precursor (Allergen Bos d 6) (BSA). n=1 Tax=Bos taurus RepID=UPI000179EC85

16a UniRef100_P04264 1.0000

confidence:	coverage:	num unique	tot indep	share of spectrum	subsumed
1.00	25.6%	peps: 16	spectra: 38	id's: 8.77%	entries: 3

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

17a UniRef100_P05787 UniRef100_Q7L4M3 UniRef100_Q969I0 **1.0000**

confidence:	max coverage:	num unique	tot indep	share of spectrum
1.00	7.9%	peps: 2	spectra: 3	id's: 0.70%

>Keratin, type II cytoskeletal 8 n=1 Tax=Homo sapiens RepID=K2C8_HUMAN Length: 483aa
>KRT8 protein n=1 Tax=Homo sapiens RepID=Q7L4M3_HUMAN
>KRT8 protein (Fragment) n=1 Tax=Homo sapiens RepID=Q969I0_HUMAN

18a UniRef100_P06576 UniRef100_Q0QEN7 **1.0000**

confidence:	max coverage:	num unique	tot indep	share of spectrum
1.00	11.2%	peps: 4	spectra: 9	id's: 2.07%

>ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB_HUMAN Length: 529aa
>ATP synthase subunit beta (Fragment) n=1 Tax=Homo sapiens RepID=Q0QEN7_HUMAN

19a UniRef100_P17676 UniRef100_Q9BSC0 **1.0000**

confidence:	max coverage:	num unique	tot indep	share of spectrum
1.00	22.6%	peps: 2	spectra: 3	id's: 0.69%

>CCAAT/enhancer-binding protein beta n=1 Tax=Homo sapiens RepID=CEBPB_HUMAN Length: 345aa
>CEBPB protein (Fragment) n=2 Tax=Eutheria RepID=Q9BSC0_HUMAN

20a UniRef100_P19474 1.0000

confidence:	coverage:	num unique	tot indep	share of spectrum	subsumed
1.00	29.9%	peps: 14	spectra: 37	id's: 8.48%	entries: 1

>52 kDa Ro protein n=1 Tax=Homo sapiens RepID=RO52_HUMAN Length: 475aa

21a UniRef100_Q562R1 1.0000

confidence:	coverage:	num unique	tot indep	share of spectrum
1.00	14.1%	peps: 5	spectra: 12	id's: 2.55%

>Beta-actin-like protein 2 n=1 Tax=Homo sapiens RepID=ACTBL_HUMAN Length: 376aa

22 UniRef100_P06872 0.9952

confidence:	coverage:	num unique	tot indep	share of spectrum
1.00	6.9%	peps: 4	spectra: 11	id's: 0.78%

>Anionic trypsin n=2 Tax=Canis lupus familiaris RepID=TRY2_CANFA Length: 247aa

23 UniRef100_A6NK07 UniRef100_B5BU01 UniRef100_P20042 UniRef100_Q4R5G5 UniRef100_Q96I16 **0.9933**

confidence:	max coverage:	num unique	tot indep	share of spectrum
0.21	5.1%	peps: 1	spectra: 2	id's: 0.47%

>Eukaryotic translation initiation factor 2 subunit 2-like protein n=1 Tax=Homo sapiens RepID=IF2BL_HUMAN Length: 327aa
>Eukaryotic translation initiation factor 2 beta n=1 Tax=Homo sapiens RepID=B5BU01_HUMAN
>Eukaryotic translation initiation factor 2 subunit 2 n=2 Tax=Homo sapiens RepID=IF2B_HUMAN
>Brain cDNA, clone: QnpA-11816, similar to human eukaryotic translation initiation factor 2, subunit 2beta, 38kDa (EIF2S2), n=1 Tax=Macaca fascicularis RepID=Q4R5G5_MACFA
>EIF2S2 protein n=1 Tax=Homo sapiens RepID=Q96I16_HUMAN

24 UniRef100_B4DEW9 UniRef100_B4DMT5 UniRef100_O00303 **0.9933**

confidence:	max coverage:	num unique	tot indep	share of spectrum
0.22	8.2%	peps: 1	spectra: 3	id's: 0.70%

>cDNA FLJ52478, highly similar to Eukaryotic translation initiation factor 3 subunit 5 n=1 Tax=Homo sapiens RepID=B4DEW9_HUMAN Length: 208aa
>cDNA FLJ52696, highly similar to Eukaryotic translation initiation factor 3 subunit 5 n=1 Tax=Homo sapiens RepID=B4DMT5_HUMAN
>Eukaryotic translation initiation factor 3 subunit F n=2 Tax=Homo sapiens RepID=EIF3F_HUMAN

25 UniRef100_B4DL86 UniRef100_B4E2U0 UniRef100_P52209 **0.9933**

confidence:	max coverage:	num unique	tot indep	share of spectrum
0.11	4.0%	peps: 1	spectra: 1	id's: 0.23%

>6-phosphogluconate dehydrogenase, decarboxylating n=1 Tax=Homo sapiens RepID=B4DL86_HUMAN Length: 429aa
>6-phosphogluconate dehydrogenase, decarboxylating n=1 Tax=Homo sapiens RepID=B4E2U0_HUMAN
>6-phosphogluconate dehydrogenase, decarboxylating n=3 Tax=Homo sapiens RepID=6PGD_HUMAN

26 UniRef100_P07954 UniRef100_P07954-2 **0.9933**

confidence:	max coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.10	3.9%	1	3	0.70%

>Fumarate hydratase, mitochondrial n=2 Tax=Homo sapiens RepID=FUMH_HUMAN Length: 510aa
>Isoform Cytoplasmic of Fumarate hydratase, mitochondrial n=1 Tax=Homo sapiens RepID=P07954-2

27 UniRef100_P22695 UniRef100_Q4R4W6 **0.9933**

confidence:	max coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.11	2.9%	1	1	0.23%

>Cytochrome b-c1 complex subunit 2, mitochondrial n=1 Tax=Homo sapiens RepID=QCR2_HUMAN Length: 453aa
>Brain cDNA, clone: QtrA-12443, similar to human ubiquinol-cytochrome c reductase core protein II(UQCRC2), n=1 Tax=Macaca fascicularis RepID=Q4R4W6_MACFA

28 UniRef100_P52597 0.9933

confidence:	coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.12	3.9%	1	3	0.70%

>Heterogeneous nuclear ribonucleoprotein F, N-terminally processed n=1 Tax=Homo sapiens RepID=HNRPF_HUMAN Length: 415aa

29 UniRef100_Q13268 UniRef100_Q13268-2 UniRef100_Q59F08 **0.9933**

confidence:	max coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.26	10.2%	1	2	0.47%

>Dehydrogenase/reductase SDR family member 2 n=3 Tax=Homo sapiens RepID=DHRS2_HUMAN Length: 258aa
>Isoform 2 of Dehydrogenase/reductase SDR family member 2 n=1 Tax=Homo sapiens RepID=Q13268-2
>Dehydrogenase/reductase (SDR family) member 2 isoform 2 variant (Fragment) n=1 Tax=Homo sapiens RepID=Q59F08_HUMAN

30 UniRef100_B4DTC3 UniRef100_B4E0W4 UniRef100_B9ZVU1 UniRef100_D6RAF8 UniRef100_D6RF44 UniRef100_Q12771 UniRef100_Q14103 UniRef100_Q14103-2 UniRef100_Q14103-3 UniRef100_Q14103-4 UniRef100_UPI0001D3B53A **0.9926**

confidence:	max coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.35	12.5%	1	3	0.70%

>cDNA FLJ54150, highly similar to Heterogeneous nuclear ribonucleoprotein D0 n=2 Tax=Eutheria RepID=B4DTC3_HUMAN Length: 303aa
>cDNA FLJ61020, highly similar to Heterogeneous nuclear ribonucleoprotein D0 n=1 Tax=Homo sapiens RepID=B4E0W4_HUMAN
>Putative uncharacterized protein HNRNPD n=1 Tax=Homo sapiens RepID=B9ZVU1_HUMAN
>Putative uncharacterized protein HNRNPD n=3 Tax=Catarrhini RepID=D6RAF8_HUMAN
>Putative uncharacterized protein HNRNPD n=2 Tax=Homo sapiens RepID=D6RF44_HUMAN
>P37 AUF1 n=1 Tax=Homo sapiens RepID=Q12771_HUMAN
>Heterogeneous nuclear ribonucleoprotein D0 n=1 Tax=Homo sapiens RepID=HNRPD_HUMAN
>Isoform 2 of Heterogeneous nuclear ribonucleoprotein D0 n=1 Tax=Homo sapiens RepID=Q14103-2
>Isoform 3 of Heterogeneous nuclear ribonucleoprotein D0 n=1 Tax=Homo sapiens RepID=Q14103-3
>Isoform 4 of Heterogeneous nuclear ribonucleoprotein D0 n=1 Tax=Homo sapiens RepID=Q14103-4
>UPI0001D3B53A related cluster n=1 Tax=Homo sapiens RepID=UPI0001D3B53A

31 UniRef100_P06733 0.9926

confidence:	coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.11	3.0%	1	3	0.70%

>Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN Length: 434aa

32 UniRef100_P23526 0.9920

confidence:	coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.11	2.5%	1	1	0.23%

>Adenosylhomocysteinase n=2 Tax=Homo sapiens RepID=SAHH_HUMAN Length: 432aa

10c UniRef100_B4DE59 UniRef100_C9JM50 UniRef100_P08727 **0.9898**

confidence:	max coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:	subsumed entries:
1.00	9.2%	1	1	0.63%	2

>cDNA FLJ60424, highly similar to Junction plakoglobin n=1 Tax=Homo sapiens RepID=B4DE59_HUMAN Length: 563aa
>Putative uncharacterized protein KRT19 n=1 Tax=Homo sapiens RepID=C9JM50_HUMAN
>Keratin, type I cytoskeletal 19 n=1 Tax=Homo sapiens RepID=K1C19_HUMAN

33 UniRef100_D3DTL2 UniRef100_D3DTL4 UniRef100_P13929 UniRef100_P13929-2 UniRef100_P13929-3 UniRef100_Q9NPL4 UniRef100_UPI0001AE669E 0.9801

confidence:	max coverage:	num unique peps:	tot indep spectra:	share of spectrum id's:
0.28	4.4%	1	1	0.23%

>Enolase n=1 Tax=Homo sapiens RepID=D3DTL2_HUMAN Length: 434aa
>Enolase n=1 Tax=Homo sapiens RepID=D3DTL4_HUMAN
>Beta-enolase n=1 Tax=Homo sapiens RepID=ENOB_HUMAN
>Isoform 2 of Beta-enolase n=1 Tax=Homo sapiens RepID=P13929-2
>Isoform 3 of Beta-enolase n=1 Tax=Homo sapiens RepID=P13929-3
>Enolase (Fragment) n=1 Tax=Homo sapiens RepID=Q9NPL4_HUMAN
>UPI0001AE669E related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE669E

34 UniRef100_UPI0000110769 UniRef100_UPI00017BDB42 **0.9590**

confidence: 0.21	max coverage: 3.3%	num unique peps: 1	tot indep spectra: 1	share of spectrum id's: 0.23%

>ANTIBODY n=1 Tax=Homo sapiens RepID=UPI0000110769 Length: 214aa

>FabOX117 Light Chain Fragment n=1 Tax=Homo sapiens RepID=UPI00017BDB42