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Datasheet for 600-401-B62S

CASZ1 Antibody

Overview

Description:	Anti-CASZ1 (RABBIT) Antibody - 600-401-B62S
Item No.:	600-401-B62S
Size:	25 µL
Applications:	ELISA, WB, ChIP, CUT&RUN, IF, IHC, Multiplex
Reactivity:	Human
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 human castor zinc finger 1 gene (CASZ1) regulates the production of the castor transcription factor, having increased expression when cells of neural and mesenchymal origin are induced to differentiation. CASZ1 is expressed in a number of human tumors and it localizes to a genomic region frequently lost in tumors of neuroectodermal origin. The CASZ1 gene generates 2 mRNA isoforms hCasz5 and hCasz11 that could encode proteins containing 5 or 11 zinc finger motifs, respectively. The proteins may be especially labile. Expect a band at 125 kDa for hCasz5 and 190 kDa for hCaz11 in fresh lysates.
Synonyms:	rabbit anti-CASZ1 Antibody, CASZ1, Zinc finger protein castor homolog 1, Castor-related protein, Zinc finger protein 693, CST, SRG, ZNF693
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	CASZ1
Reactivity:	Human
Immunogen Type:	Conjugated Peptide

Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an internal region of Human Casz1 protein.
Purity/Specificity:	This affinity purified antibody is directed against human Casz1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest reactivity with CASZ1 proteins from human, mouse, Drosophila, chimpanzee, and macaque based on a 100% homology. Partial reactivity is expected with horse and dog CASZ1 based on a 92% homology with the immunizing sequence. Cross-reactivity with CASZ1 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q86V15 • NCBI - 145207289 • GeneID - 54897

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	ChIP, CUT&RUN, IF, IHC, Multiplex (Based on references)
Application Note:	This antibody has been tested for use in ELISA, IHC, IF, and western blotting. This antiserum detects endogenous Casz1 proteins, both the hCasz5 and hCasz11 isoforms. Expect a band approximately 125 kDa for hCasz5 and 190 kDa for hCaz11 in size corresponding Casz1 by western blotting in the appropriate cell lysate or extract. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:45,000
IF:	User Optimized
IHC:	User Optimized
WB:	1:1,000 - 1:5,000

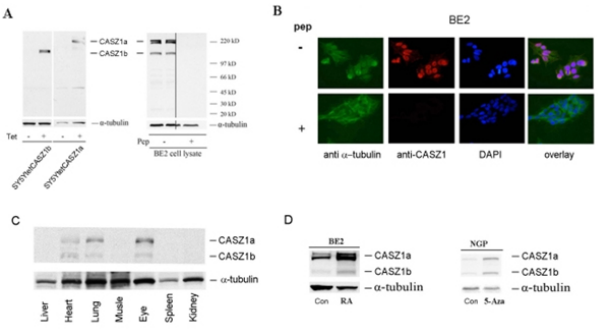
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.97 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

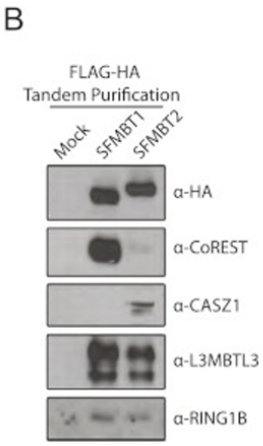
Shipping & Handling

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.

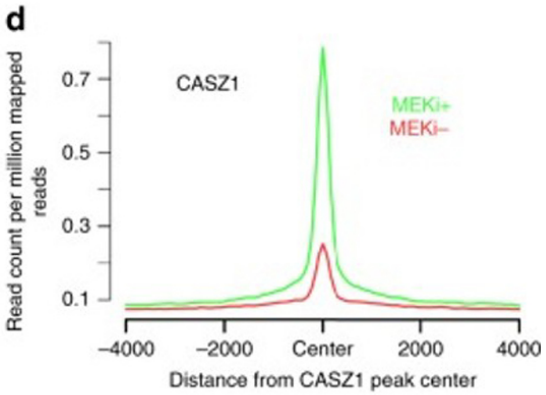
Images



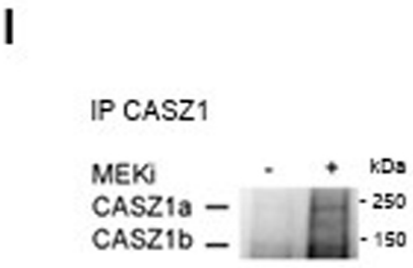
Immunofluorescence Microscopy
A. Anti-CASZ1 antibody is specific to CASZ1a and CASZ1b. Induction of CASZ1a and CASZ1b expression in the SY5YtetCASZ1a and SY5YtetCASZ1b clones by Tet at 24 hours was visualized by immunoblotting the cell lysates with anti-CASZ1 antibody (left); endogenous CASZ1a and CASZ1b expression in neuroblastoma cells was visualized by immunoblotting the BE2 cell lysates with anti-CASZ1 antibody, and the specificity of the antibody was demonstrated by pre-incubation of immunizing peptide and loss of CASZ protein recognition with immunoblotting analysis (right). B. Endogenous CASZ1a and CASZ1b are predominantly expressed in the nucleus of neuroblastoma cells. The nuclear localization of CASZ1a and CASZ1b was characterized by the co-localization of Alex 568-labeled goat anti-mouse IgG and DAPI-stained nucleus of BE2 cells (top), and the specificity of the antibody was demonstrated by the blocking effect of antigen peptide on antibody recognition with immunoblotting analysis (bottom). C. The co-expression of murine CASZ1a and CASZ1b protein in heart, lung and eye of P6 mouse was visualized by immunoblot analysis of tissue lysates using an anti-CASZ1 antibody. D. The simultaneous up-regulation of CASZ1a and CASZ1b protein by either RA or 5-Aza-dC in neuroblastoma cell lines was visualized by immunoblot analysis of the cell lysates using the anti-CASZ1 antibody. Fig 3. PMID: 21490919



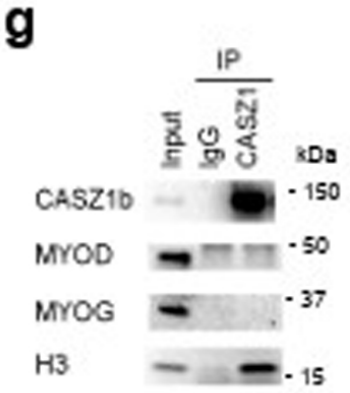
Western Blot
(B) Western blot analysis of specific associated polypeptides using elutions from TAPs. Fig 1. PMID: 23592795



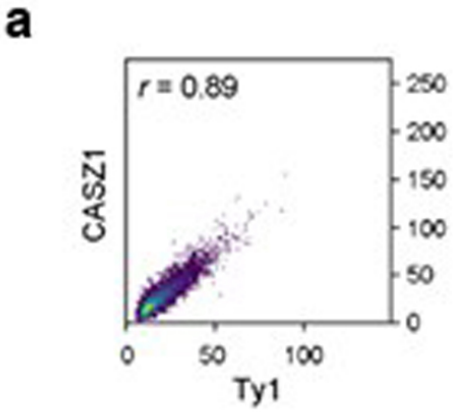
ChIP
d ChIP-seq was performed using anti-CASZ1 antibody in SMS-CTR cells with or without MEKi treatment. Composite plot shows the increase of endogenous CASZ1 binding on genomic DNA after SMS-CTR cells are treated with 100 nM MEKi for 48 h. Fig 2. PMID: 32060262



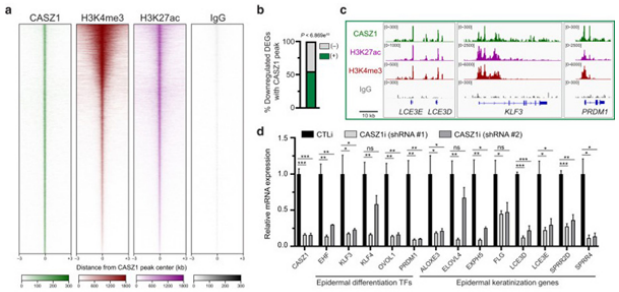
Immunoprecipitation
I, Immunoprecipitation of CASZ1 using anti-CASZ1 antibody from formaldehyde cross-linked control or MEKi-induced, differentiated SMS-CTR cells showed the pulldown of CASZ1a and CASZ1b. SF2. PMID: 32060262



Immunoprecipitation
g, Co-immunoprecipitation in CASZ1b restored SMS-CTR cells using anti-CASZ1 antibody showed the pulling down of a known CASZ1b protein partner histone 3 (H3), but not MYOD or MYOG. SF4. PMID: 32060262

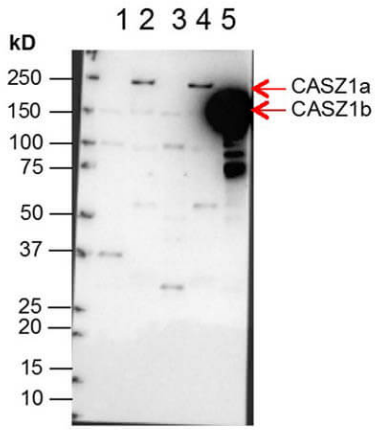


Flow Cytometry
a, In 3xTy1 tagged CASZ1b restored SMS-CTR cells, ChIP-seq data got from anti-Ty1 antibody and anti-CASZ1 antibody shows significant Pearson correlation. SF5. PMID: 32060262



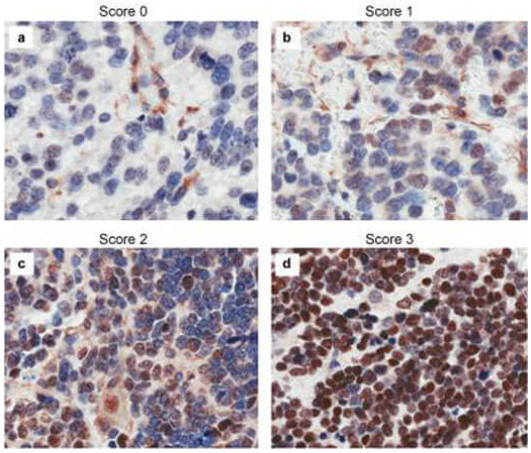
Figure

CASZ1 binds to promoter regions of epidermal differentiation genes and regulates their expression. (a) Heatmaps showing CUT&RUN read densities for CASZ1, H3K4me3, H3K27ac, and IgG across CASZ1 genomic peaks in differentiated KCs. (b) Bar graph showing the percentage of DEGs downregulated in CASZ1i that are bound by CASZ1 in control differentiated KCs. (c) IGV browser views showing CUT&RUN tracks of CASZ1, H3K4me3, H3K27ac, and IgG for indicated epidermal differentiation genes. (d) RT-qPCR analysis of a panel of epidermal differentiation genes in CASZ 1i and CTLi differentiated KCs. Two separate shRNA sequences (shRNA#1 and shRNA#2) targeting different regions of the CASZ1 gene were used. Data are presented as mean ± SEM, n = 3; ⍰P < .05, ⍰⍰P < .01, and ⍰⍰⍰P < .001 (unpaired 2-tailed t-test). CASZ1i, silenced CASZ1; CTLi, silenced control; CUT&RUN, cleavage under targets & release using nuclease; DEG, differentially expressed gene; IGV, Integrated Genomics Viewer; KC, keratinocyte; ns, not significant; shRNA, short hairpin RNA; TF, transcription factor. Fig 2. PMID: 38763175

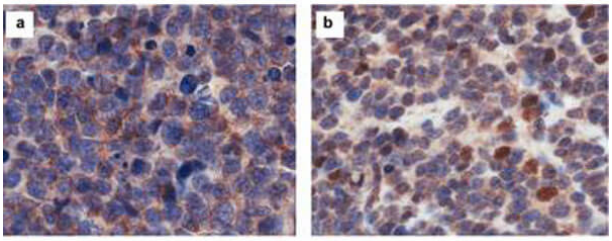


Western Blot

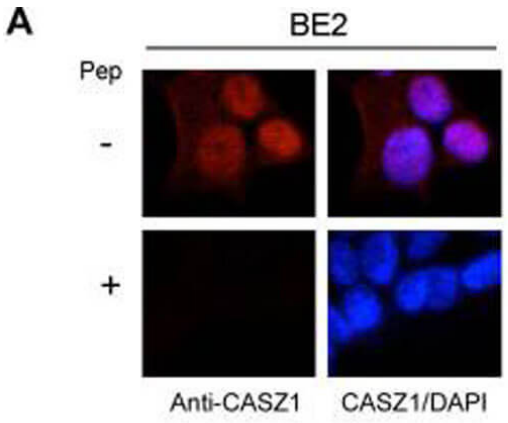
Western Blot of Anti-CASZ1 Antibody. Lane 1: NBLS Cytoplasmic (20µg). Lane 2: NBLS Nuclear (3µg). Lane 3: BE2C Cytoplasmic (30µg). Lane 4: BE2C Nuclear (7µg). Lane 5: SY5Y-CASZ1b (10µg). Block: 5% Blotto/TTBS for 1 hour. Primary: Casz1 1:10,000 for 1 hour. Secondary: Goat anti-Rabbit HRP for 1 hour. 240sec exposure. Detects nuclear endogenous CASZ1a and CASZ1b; and transiently transfected CASZ1b isoform. Personal communication and images from Carol Thiele Galetto, NCI.



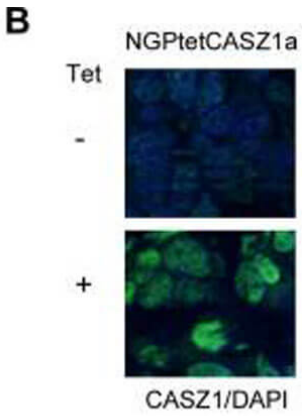
Immunohistochemistry
(B) Examples of nuclear staining: a. Score 0 - a rare positive nuclei; b. Score 1 (1–10% positive) equivocal/uninterpretable; c. Score 2 (10–50%) weak positive, or d. Score 3 (> 50% positive cells)-strong positive. Fig 9. PMID: 27270431



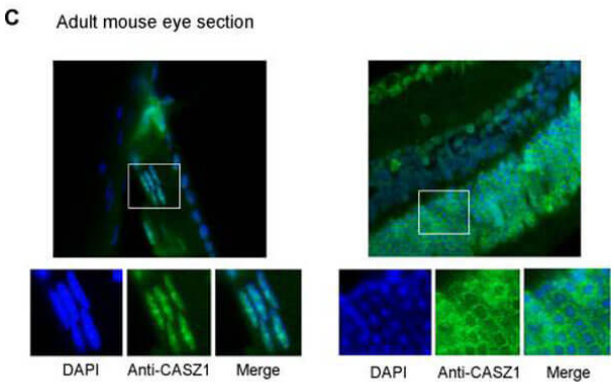
Immunohistochemistry
(A) a. CASZ1 localized exclusively in the cytoplasm showed by staining the tumor samples with anti-CASZ1 antibody; b. CASZ1 localized to both the nucleus and the cytoplasm. Nucleus was counterstained with hematoxylin solution showed in blue, and CASZ1 was probed with rabbit anti-CASZ1 antibody and showed in brown. Fig 9.
PMID: 27270431



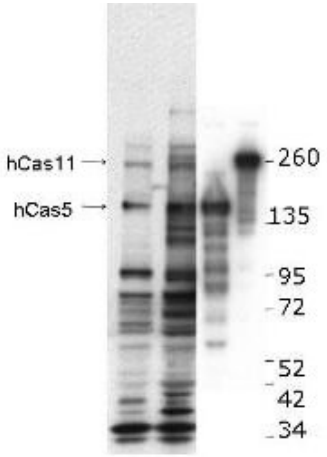
Immunofluorescence Microscopy
(A) Endogenous CASZ1 subcellular localization in BE2 cells was determined by staining the cells with rabbit anti-CASZ1 antibody. The anti-CASZ1 antibody was pre-incubated with (bottom panel) or without (top panel) CASZ1 antigen peptide (Pep) that used to generate CASZ1 antibody. The chromatin was stained with DAPI. Fig 8. PMID: 27270431



Immunofluorescence Microscopy
(B) Mouse xenograft tumor of human NB cell line that has been stably transfected with tetracycline inducible CASZ1 (NGPtetCASZ1) was dissected and stained with rabbit anti-CASZ1 antibody and DAPI. Fig 8. PMID: 27270431



Immunofluorescence Microscopy
(C) Endogenous CASZ1 in the eye of adult mouse was stained with rabbit anti-CASZ1 antibody and DAPI. Localization: nucleus in lens epithelia but primarily localizes in the cytoplasm in photoreceptor cells. Fig 8. PMID: 27270431



Western Blot
Western blot using Rockland's anti-hCASZ1 antibody. This blot shows detection of endogenous and transfected human CASZ1 protein in fresh whole cell lysate (~30 µg). Lane 1: BE2(s) cell lysate. Lane 2: BE2(N) cell lysate. Lane 3: SY5Y transfected with hCASZ5 (125kDa). Lane 4: SY5Y transfected with hCASZ11 (190kDa). Protein was resolved by SDS-PAGE and transferred onto nitrocellulose. After blocking, the membrane was probed with the primary antibody diluted to 1:1,000 for 1.5 hours at room temperature then incubated with HRP-conjugated Goat Anti-Rabbit antibody for 45 min. at room temperature. Personal communication, Carol Thiele, NCI, Bethesda, MD.

References

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