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Datasheet for 600-401-270

CREB phospho S133 Antibody

Overview

Description:	Anti-CREB pS133 (RABBIT) Antibody - 600-401-270
Item No.:	600-401-270
Size:	100 µL
Applications:	ELISA, IHC, WB
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

Product Details

Background:	The CREB (Cyclic AMP-response-element-binding-protein 1) gene encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds as a homodimer to the cAMP-responsive element (CRE element TGANNTCA), an octameric palindrome. Phosphorylation by cAMP-dependent protein kinase (PKA) at serine-119 is required for interaction with DNA and phosphorylation at serine-133 allows CREB to interact with CBP (CREB binding protein) leading to interaction with RNA polymerase II. Alternate splicing of this gene results in two transcript variants encoding different isoforms.
Synonyms:	rabbit anti-CREB pS133 Antibody, Cyclic AMP-responsive element-binding protein 1, Active transcription factor CREB antibody, cAMP responsive element binding protein 1 antibody, CREB 1 antibody, CREB-1, CREB1 antibody, MGC9284 antibody, Transactivator protein antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	CREB1
Reactivity:	Human, Mouse, Rat
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide

Immunogen:	CREB phospho peptide corresponding to amino acid residues 122-147 of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	Anti-CREB pS133 was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first sequentially pre-adsorbed against a control E.coli lysate, nickel-purified recombinant CREB and the non-phosphorylated form of the immunizing peptide. The resultant depleted antiserum was then purified against the phosphorylated form of the immunizing peptide. This phospho specific polyclonal antibody is specific for phosphorylated pS133 of human CREB. Reactivity with non-phosphorylated CREB is minimal. This antibody does show cross reactivity with pS133 phosphorylated CREB from mouse and rat.
Relevant Links:	• NCBI - CAG28545.1 • UniProtKB - P16220 • GeneID - 1385

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	Anti-CREB pS133 antibody reacts with phosphorylated human CREB at pS133 and shows minimal reactivity by western blot with non-phosphorylated CREB and minimal reactivity (1%) by ELISA against the non-phosphorylated form of the immunizing peptide. This antibody was assayed against a variety of tissues including fibroblasts and B-cell lysates. Bands of 46 and 43 kDa corresponding to phosphorylated CREB are observed in western blots. Anti-CREB pS133 is suitable for the detection by immunoblot of phosphorylated human, mouse and rat CREB. No cross-reactivity occurs with non-phosphorylated CREB. For immunohistochemistry, formalin fixed, paraffin embedded human tissue shows moderate to strong nuclear staining in a variety of cells with minimal background staining. Although not tested this antibody is likely functional by FACS and immunoprecipitation.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:1,000 - 1:6,000
IHC:	20 µg/ml
WB:	1:500 - 1:2,000

Formulation

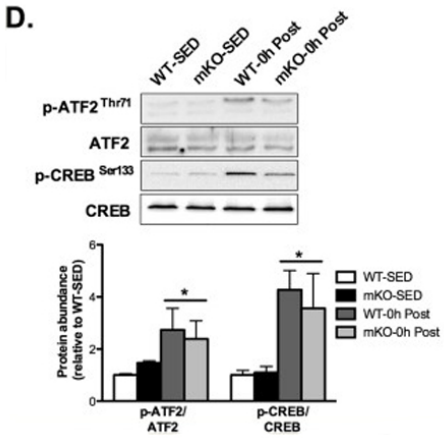
Physical State:	Liquid (sterile filtered)
Concentration:	0.064 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

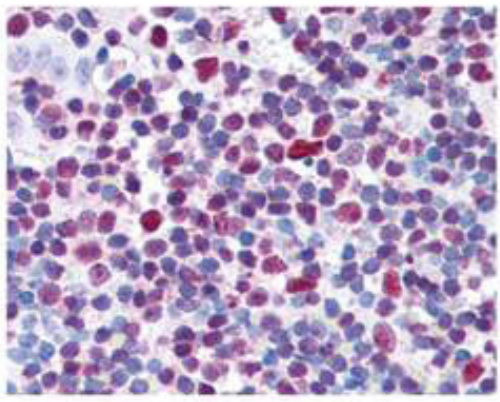
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

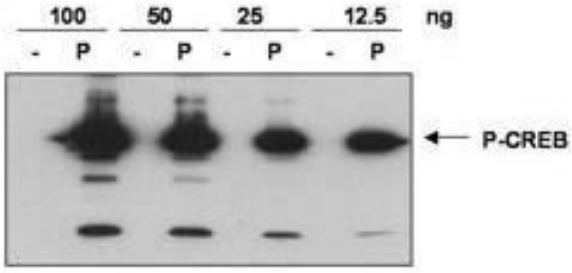
Images



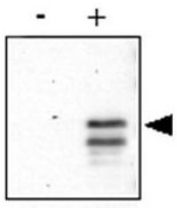
Western Blot
mKO mice display normal activation of exercise-responsive proteins following AEX. Protein phosphorylation and total abundance were determined in gastrocnemius whole muscle lysates of sedentary (SED) mice or immediately after AEX (0 h post).
D. Phosphorylation of ATF2Thr71 and CREBSer133. Data presented as mean ± S.E. (error bars). *, within genotype $p < 0.05$.
FIGURE 3. PMID: 21757760.



Immunohistochemistry
Rockland's affinity purified anti-CREB pS133 antibody was used at 20µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate to strong nuclear staining of tonsillar lymphocytes. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.



Western Blot
Anti-CREB pS133 was used to detect phosphorylated CREB by western blot. Recombinant His-tagged human CREB was produced in E.coli and purified by metal affinity chromatography. An aliquot of purified CREB was phosphorylated in-vitro using Protein Kinase A and ATP. Western blot of indicated amounts (100ng, 50ng, 25ng, 12.5ng) of control (-) and in-vitro phosphorylated CREB (P) were loaded to show that the antibody reacts specifically with the phosphorylated form. Blots were blocked in 5% milk in TBS+0.1% Tween-20 (TBST-M) overnight at 4°C. Detection occurs using a 1:500 dilution of antibody diluted in TBST-M and incubated at room temperature with rocking for 1 hour. Blots were rinsed 6X with TBST and incubated with goat anti-rabbit-HRP at 1:5000 in TBST-M at room temperature for 45 min. Blots were again rinsed 6X with TBST and then processed using ECL reagent (Amersham) according to manufacturer's instructions. Exposure time: 1 min using Kodak Biomax MR film. Personal Communication, R. Screaton, The Salk Institute for Biological Studies.



Western Blot
Anti-CREB pS133 was used to detect phosphorylated CREB by western blot at ~46kDa. Recombinant His-tagged human CREB was produced in E.coli and purified by metal affinity chromatography. An aliquot of purified CREB was phosphorylated in-vitro using Protein Kinase A and ATP. Western blot of control (-) and in-vitro phosphorylated CREB (+) was used to show that the antibody reacts specifically with the phosphorylated form. Pan reactive CREB (Rockland # 100-401-195) reacts equally with both non-phosphorylated and phosphorylated CREB (not shown). Detection occurs using a 1:500 dilution of antibody followed by 1:5,000 dilution of HRP Goat-a-Rabbit IgG with visualization via ECL. Film exposure was approximately 1'. Other detection systems will yield similar results. Personal Communication, Boss, J., Emory University School of Medicine, Atlanta, GA.

References

- Perez-Schindler J et al. Regulation of contractility and metabolic signaling by the β2-adrenergic receptor in rat ventricular muscle. *Life Sci.* (2011)
- Philp A, Chen A, Lan D, et al. Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) deacetylation following endurance exercise. *J Biol Chem.* (2011)
- Reiners J et al. A polymorphism linked to bipolar affective disorder does not alter the CRE activity of constitutively activated trace amine receptor 4. *Mol Psychiatry.* (2007)

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