ENCODE DCC Antibody Validation Document

Date of Submission: 1/4/12

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Antibody Name: CoREST (H-65) Antibody: ab24166

Target: COREST (EntrezGene ID 23186)

Company/ Source: Abcam

Catalog Number, database ID, laboratory: ab24166

Lot Number: 834865

Antibody Description: Synthetic peptide conjugated to KLH derived from within residues 400 to the C-terminus of Human CoREST.
Note: This antibody has since been discontinued. Full product information is attached at the end of this document.

Target Description: Essential component of the BHC complex, a corepressor complex that represses transcription of neuron-specific
genes in non-neuronal cells. The BHC complex is recruited at RE1/NRSE sites by REST and acts by deacetylating and

Species Target: Human

Species Host: Rabbit

Validation Method #1: Western Blot

Validation Method #2: ChIP

Purification Method: Affinity

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: NOT AVAILABLE- ANTIBODY DISCONTINUED;

Reference (PI/ Publication Information):

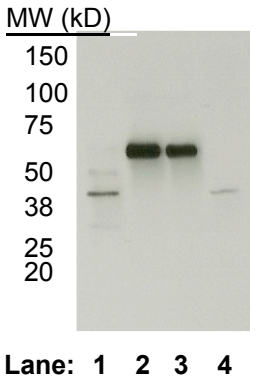
Please complete the following for antibodies to histone modifications:
if your specifications are not listed in the drop-down box,
please write-in the appropriate information

Histone Name | AA modified | AA Position | Modification

Validation #1 Analysis	COMMENT: We observe a single band in Western blots on cell lines K562, HeLa S3, and HepG2 that migrates slightly slower than the predicted size (~53kD) would suggest. This has been previously observed (see product literature for this and other commercial antibodies, as well as the Western blot assays in our characterization document for ab24166 against COREST). This band, but not the observed weaker bands, is specifically immunoprecipitated by sc-30189. Based on this, sc-30189 is validated by this criterion.

Insert Validation Image (click here)

Antibody ab24166 (CORREST)
Immunoblot



Western blot using antibody ab24166 on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4).

Validation #2 Analysis	Comment: Because the overlap of the top 40% of peaks from datasets derived from antibodies sc30189 and ab24166 (compared using standard ENCODE scoring methods) exceeds the recommended threshold of 80%, both antibodies meet this criterion for validation.

Insert Validation Image (Click here)

Submit by Email

Validation 2: ChIPseq with alternate antibodies to the same factor

	COREST sc30189	COREST ab24166
Total peaks	111692	30269
% Peak overlap	**87.6**	**93.2**

Antibodies:
sc30189: epitope corresponding to amino acids 246-310 within CoREST of human origin
ab24166: immunogen is synthetic peptide conjugated to KLH derived from resides 400 to C-terminus of human COREST.

Comparison: K562 cells were used for ChIP-seq with antibody sc-30189 or antibody ab24166. Peaks were called from replicate experiments using PeakSeq with a .01 q-value cut-off. Comparisons between experiments were made using these peaks according to standard ENCODE replicate comparison parameters (http://genome.ucsc.edu/ENCODE/protocols/dataStandards/ChIP_DNase_FAIRE_DNAme_v2_2011.pdf; reported is the fraction of the top 40% of peaks in one list that are found in the full list of peaks obtained with the other antibody.

Rabbit polyclonal to CoREST - ChIP Grade · (ab24166)

Printed on 30 December 2011

Product Constituents and Storage

Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4 This product may contain a hazardous substance. For a material safety datasheet (MSDS) please see our website for a downloadable PDF. Please let us know if you are unable to access the Internet and we will either send you a hard copy MSDS or fax the relevant MSDS to you at your request.
Form	Liquid
Concentration	0.50 mg/ml
Storage instructions	Store at +4℃ short term (1-2 weeks). Aliquot and store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Product Description

Immunogen	Synthetic peptide conjugated to KLH derived from within residues 400 to the C-terminus of Human CoREST. (Peptide available as ab25740.)
Species Reactivity	Reacts with Human. Predicted to react with Mouse (88% identity with immunogen) due to sequence homology.
Target	Information below.
Function	Essential component of the BHC complex, a corepressor complex that represses transcription of neuron-specific genes in non-neuronal cells. The BHC complex is recruited at RE1/NRSE sites by REST and acts by deacetylating and demethylating specific sites on histones, thereby acting as a chromatin modifier. In the BHC complex, it serves as a molecular beacon for the recruitment of molecular machinery, including MeCP2 and SUV39H1, that imposes silencing across a chromosomal interval. Plays a central role in demethylation of Lys-4 of histone H3 by promoting demethylase activity of KDM1A on core histones and nucleosomal substrates. It also protects KDM1A from the proteasome.
Tissue specificity	Ubiquitously expressed.
Sequence similarities	Belongs to the CoREST family. Contains 1 ELM2 domain. Contains 2 SANT domains.
UniProt	Target information above from: UniProt accession Q9UKL0
Clonality	Polyclonal
Isotype	IgG
Purity	Immunogen affinity purified

Product Use

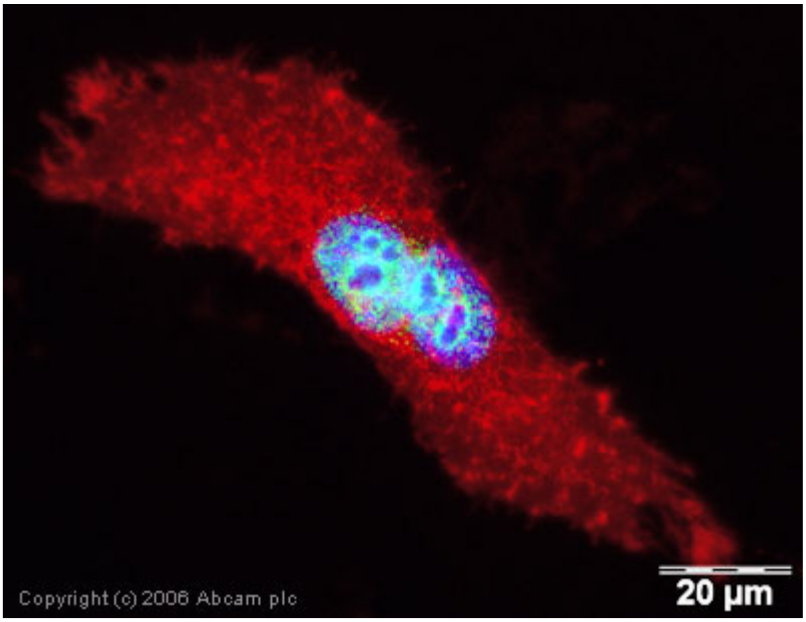
Application notes	**Recommended dilutions** IHC-P: Use at a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. ChIP: Use at a concentration of 3 µg/ml. ICC/IF: Use at a concentration of 5 µg/ml. WB: Use at a concentration of 1 µg/ml. Detects a band of approximately 76 kDa (predicted molecular weight: 70 kDa). Can be blocked with CoREST peptide (ab25740).

Not yet tested in other applications.

Optimal dilutions/concentrations for use should be determined by the end user.

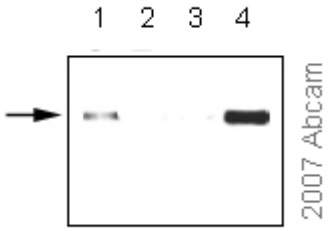
Positive Control Images

Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
See www.abcam.com/ab24166 for more detail.



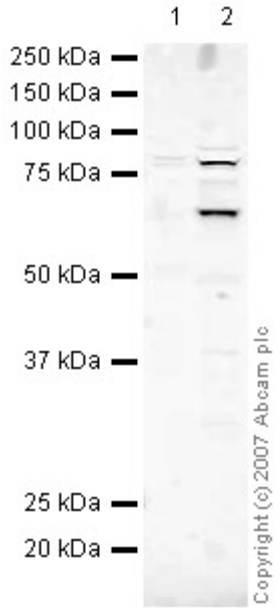
ICC/IF

ICC/IF image of ab24166 stained human HeLa cells. The cells were PFA fixed (3.7% PFA, 10 min) and incubated with the antibody (ab24166, 5µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT^{TM} FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



ChIP

PCR was performed on a SCNA1 promoter following CoRest chromatin immunoprecipition using ab24166 at a concentration of 3ug/ml from 293T cells.
Lane 1 - ab24166
Lane 2 - Beads only control
Lane 3 - IgG only control
Lane 4 - Input



WB

All lanes : Anti-CoREST antibody - ChIP Grade (ab24166) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

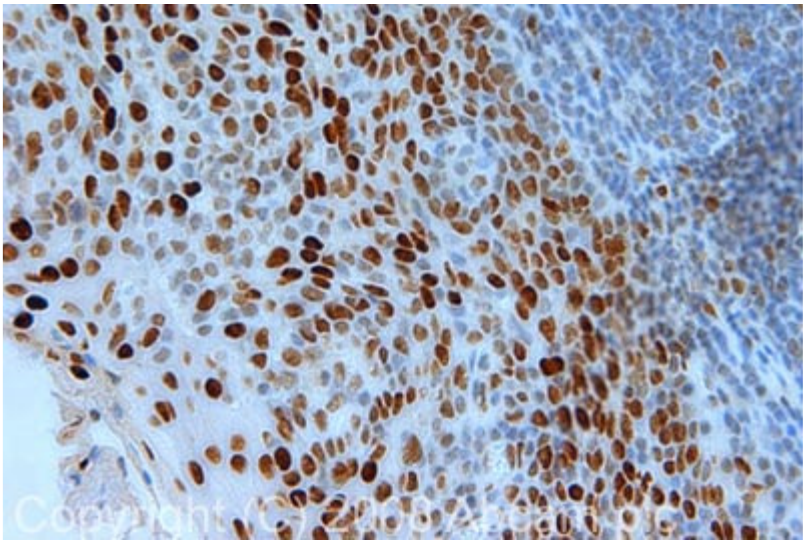
Lysates/proteins at 10 µg per lane.

Secondary
IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size : 70 kDa
Observed band size : 76 kDa (why is the actual band size different from the predicted?)
Additional bands at : 66 kDa. We are unsure as to the identity of these extra bands.

The observed band at 76 kDa is similar to the migration pattern seen by other commercially available antibodies to this target. Publications also support this data (María E. Andrés et al, 1999, PMID 10449787).



IHC-P

IHC image of CoREST staining in human tonsil FFPE section, performed on a Bond^{TM} system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab24166, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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