# ENCODE DCC Antibody Validation Document

Date of Submission: 1/4/12

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Antibody Name: CoREST (H-65) Antibody: sc-30189

Target: COREST (EntrezGene ID 23186)

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-30189

Lot Number: C0806

Antibody Description: epitope corresponding to amino acids 246-310 mapping within an internal region of CoREST of human origin
recommended for detection of CoREST of mouse, rat and human origin

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: Not Applicable (control)

Validation Method #1: Immunoprecipitation

Validation Method #2: ChIP

Purification Method: Other Purification Method

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-30189-cores

Reference (PI/ Publication Information):

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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 sc-30189 (COREST) Immunoblot/immunoprecipitation


**A.** Western blot using antibody sc-30189 on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4). **B.** Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody sc-30189. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with sc-30189. Lane 3: Bound material from immunoprecipitation with sc- sc-30189. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (18kD) that is highly enriched in the specifically immunoprecipitated fraction.

| | |
|---|---|
| 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)

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# 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.