

Broad Institute - Encode3 Secondary Antibody Validation

Abcam ab109398 Lot YJ082421DS

Target: EZH2 phospho-Thr487

Approved name: enhancer of zeste 2 polycomb repressive complex subunit 2

Function: EZH2 is the catalytic subunit of the polycomb related complex PRC2. It functions as the methyltransferase which writes the methyl mark on Histone H3 Lysine 27. EZH2 phosphorylation by CDK1 at Thr487 may promote cell cycle regulated, ubiquitination mediated, degradation of EZH2. In chromatin prepared from asynchronous cultures, the genomewide distribution of H3K27me3 and EZH2 phospho-Thr487 show a very high degree of correlation.

Member of complex: EZH2 is a component of the PRC2 complex, which is a chromatin modifier that aids in the maintenance of transcriptional silencing.

Other complex members: EED, EZH1, RbBP4, RbBP7, SUZ12

References:

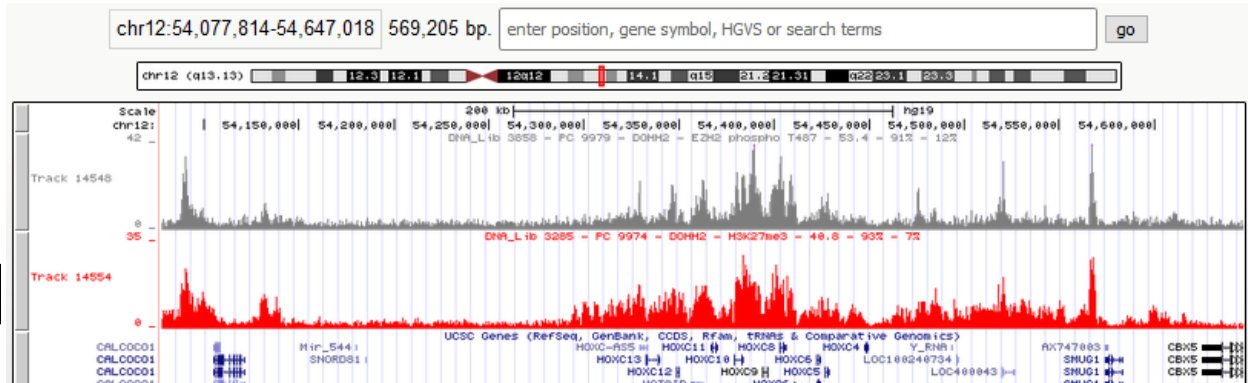
1. Genes Dev. 2002. **16**:2893-2905. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. [PMID:12435631](#).
2. Nature Cell Biology 2011 **13**, 87–94. CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. Wei Y, Chen Y-H, Li L-Y, Lang J, Yeh S-P, Shi B, Yang C-C, Yang J-Y, Lin C-Y, Lai C-C, Hung M-C. [PMID: 21131960](#).

Antibody being validated:

1. Abcam ab109398 Lot: YJ082421DS [Rabbit monoclonal, affinity purified]
2. Broad Alias: PchAb 919
3. Immunogen: Phospho specific peptide corresponding to residues surrounding threonine 487 of Human KMT6/EZH2 (Q15910)
4. <https://www.encodeproject.org/antibodies/ENCAB000BKV/>

This validation relies on the use of antibodies to a chromatin regulator and a functionally related histone modification in DOHH2 cells, and the demonstration that highly similar patterns of enrichment are obtained with each antibody. The first track shown used an antibody to phosphorylated EZH2 (PchAb 919, ENCAB000BKV), and the second track shown used an antibody to H3K27me3 previously characterized to Encode standards (PchAb 683, ENCAB000BLA).

Overall correlation: >0.85



Antibody being compared:

1. Millipore 07-449 Lot:2039786 [Rabbit polyclonal, Protein A purification]
2. Broad Alias: PchAb 683
3. Immunogen: KLH-conjugated, synthetic 2X-branched peptide containing sequenceAR(me3K)SAP.... in which me3K corresponds to trimethyl-lysine at residue 27 of human histone H3
4. <https://www.encodeproject.org/antibodies/ENCAB000BLA/>