Broad Institute - Encode3 Secondary Antibody Validation

Active Motif 39639 Lot: 10310002

Target: EZH2

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

<u>Function</u>: EZH2 is the catalytic subunit of the polycomb related complex PRC2. It functions as the methyl transferase which writes the methyl mark on Histone H3 Lysine 27. Consequently, there is a highly correlated pattern in the genomewide distribution of H3K27me3 and the phosphorylated derivative of EZH2, or phopho-EZH2, the protein form recognized by this antibody.

<u>Member of complex</u>: 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

Reference:

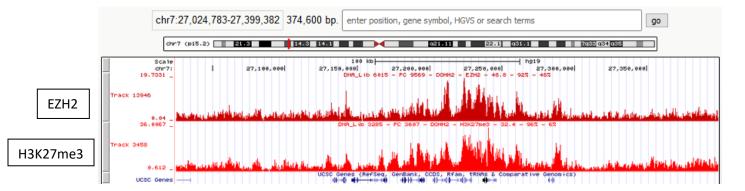
1. Genes Dev. 2002. **16**:2893-2905. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. PMID:12435631.

Antibody being validated:

- 1. Active Motif 39639 Lot: 10310002 [Rabbit polyclonal, affinity purified]
- 2. Broad Alias: PchAb 57-V
- 3. Immunogen: Recombinant fusion protein corresponding to amino acids 1-370 of mouse EZH2
- 4. https://www.encodeproject.org/antibodies/ENCAB127VPP/

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 57-V, ENCAB127VPP), and the second track shown used an antibody to H3K27me3 previously characterized to Encode standards (PchAb 683, ENCAB000BLA).

Genomewide correlation: >0.9



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/