

Recombinant PRC2 EZH2(Y641C) complex

Catalog No: 31389, 31789

Expressed In: Baculovirus

Quantity: 20 µg

Concentration: 1.2 µg/µl

Source: Human

Buffer Contents: Full length recombinant PRC2 EZH2(Y641C) Complex expressed in Sf9 at a concentration of 1.2 µg/µl in 25 mM Tris pH 7.4, 300mM NaCl, 5% Glycerol, 0.04% Triton X-100, 0.2 mg/ml 3x FLAG peptide.

Background: PRC2 (Polycomb Repressive Complex 2) is one of the two classes Polycomb-group, or PcG proteins (the other being PRC1) that are important epigenetic determinants of stem cell identity. They play an important role in long-term epigenetic silencing of genes during cell fate determination and differentiation. PRC2 functions as a repressor of chromatin. PRC2 is required to target recruitment to specific DNA sequences (termed Polycomb Response Elements or PREs) of genomic regions to be silenced. Once associated with chromatin, the PRC2 subunit EZH2 has histone methyltransferase activity that catalyzes the trimethylation of histone H3 at Lys27. H3K27me3 is well established as a hallmark of regions of repressed chromatin. Trimethylation of Lys27 leads to the recruitment of PRC1 through the binding of H3K27me3 by chromodomain-containing proteins in PRC1. PRC1 is responsible for long-term gene silencing after cellular differentiation.

Recombinant PRC2 EZH2(Y641C) Complex is a mutant version of our wild-type PRC2 Complex that contains a Tyr-to-Cys mutation at tyrosine 641 of the SET domain of EZH2. Somatic mutations of tyrosine 641, including Y641F, Y641N, Y641C, Y641S and Y641H, have been identified in patients with non-Hodgkin lymphoma and have been shown to alter substrate specificity and catalytic activity of EZH2 for histone H3 lysine 27 (H3K27) methylation states resulting in increased H3K27 trimethylation.

Protein Details: Recombinant PRC2 EZH2 (Y641C) Complex that includes full length EZH2 with a Y641C mutation complexed with full length SUZ12, EED and RbAp46/48 (accession numbers NP_001190176.1, NP_056170, NP_003788.2, NP_002884.1, and NP_005601.1, respectively) was expressed in Sf9 and contains an N-terminal FLAG tag at the N-terminus of EZH2. The molecular weights of expressed EZH2 (Y641C), SUZ12, EED and RbAp46/48 are 87 kDa, 83 kDa, 50.2 kDa, 47.8 kDa and 47.7 kDa, respectively. The recombinant protein is >92% pure by SDS-PAGE.

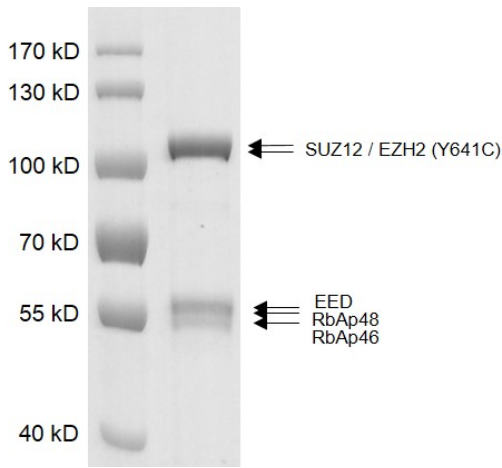
Application Notes: PRC2 EZH2(Y641C) Complex is suitable for use in the study of enzyme kinetics, inhibitor screening and selectivity profiling.

Specific Activity: H3K27me3 and H3K27me2 methyltransferase.

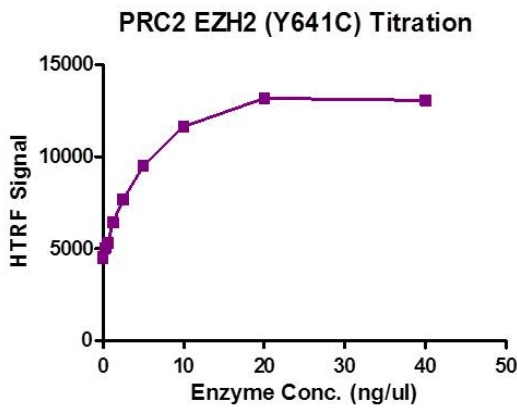
HMT Assay Conditions: 1 µM H3K27me2 peptide was incubated with different concentration of PRC2 EZH2 (Y641C) Complex in reaction buffer including 50 mM TrisCl, pH 8.6, 0.02% Triton X-100, 2 mM MgCl₂, 1 mM TCEP, 20 µM SAM for 3 hours at room temperature. Activity was detected by HTRF assay and MALDI-TOF.

Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is guaranteed for 6 months from date of receipt.

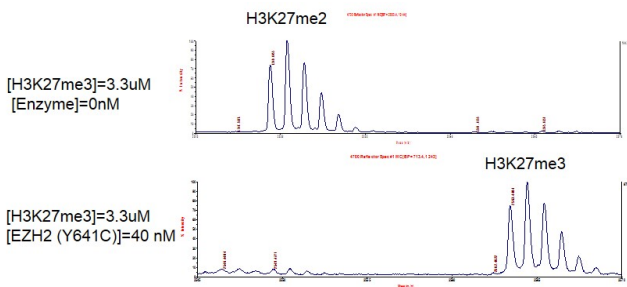
This product is for research use only and is not for use in diagnostic procedures.



Recombinant PRC2 EZH2(Y641C) Complex gel.
 PRC2 EZH2(Y641C) Complex was run on a 10% SDS-PAGE gel and stained with Coomassie Blue.



Recombinant PRC2 EZH2(Y641C) Complex activity assay.
 1 μ M H3K27me2 peptide was incubated with different concentration of PRC2 EZH2(Y641C) Complex in reaction buffer for 3 hour at room temperature. PRC2 EZH2(Y641C) Complex was used in a HTRF assay to determine enzyme linearity. Methylated peptide (H3K27me3) was measured using H3K27me3-specific antibody.



Recombinant PRC2 EZH2(Y641C) Complex activity assay.
 PRC2 EZH2(Y641C) Complex was used in an HMT assay to determine enzyme activity. Activity was detected by MALDI-TOF.

Catalytic Ability: 80 turnovers / enzyme molecule.