

## Anti-KMT6/EZH2 Antibody Picoband®

Catalog Number: A00050-1

### About EZH2

Enhancer of zeste homolog 2 (EZH2) is a histone-lysine N-methyltransferase enzyme encoded by EZH2 gene. It is mapped to 7q36.1. This gene encodes a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations. This protein associates with the embryonic ectoderm development protein, the VAV1 oncoprotein, and the X-linked nuclear protein. This protein may play a role in the hematopoietic and central nervous systems. Multiple alternatively spliced transcript variants encoding distinct isoforms have been identified for this gene.

### Overview

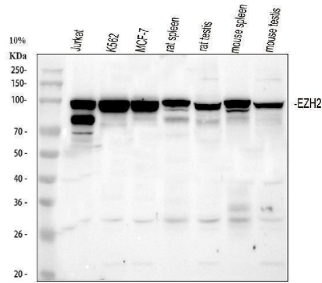
Product Name	Anti-KMT6/EZH2 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-KMT6/EZH2 Antibody Picoband® catalog # A00050-1. Tested in ELISA, Flow Cytometry, IP, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IP, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q15910

### Technical Details

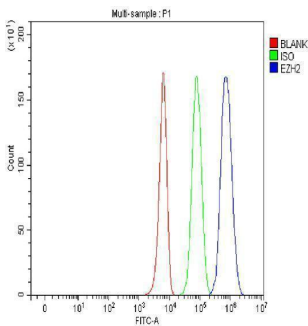
Immunogen	E.coli-derived human KMT6/EZH2 recombinant protein (Position: E22-T345).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml Immunoprecipitation, 0.5-2 ug/ml Flow Cytometry(Fixed), 1-3 ug/1x10 <sup>6</sup> cells ELISA, 0.1-0.5ug/ml

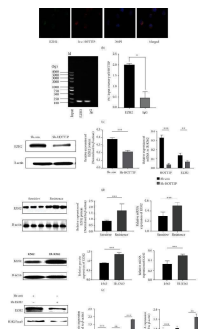
## Anti-KMT6/EZH2 Antibody Picoband® (A00050-1) Images



Western blot analysis of KMT6/EZH2 using anti-KMT6/EZH2 antibody (A00050-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human Jurkat whole cell lysates, Lane 2: human K562 whole cell lysates, Lane 3: human MCF-7 whole cell lysates, Lane 4: rat spleen tissue lysates, Lane 5: rat testis tissue lysates, Lane 6: mouse spleen tissue lysates, Lane 7: mouse testis tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-KMT6/EZH2 antigen affinity purified polyclonal antibody (Catalog # A00050-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for KMT6/EZH2 at approximately 98 kDa. The expected band size for KMT6/EZH2 is at 85 kDa.

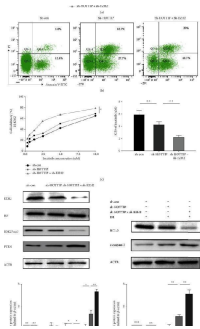


Flow Cytometry analysis of K562 cells using anti-KMT6/EZH2 antibody (A00050-1). Overlay histogram showing K562 cells stained with A00050-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-KMT6/EZH2 Antibody (A00050-1, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

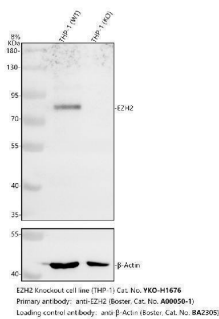


HOTTIP regulates EZH2 to inhibit PTEN expression. Note: (a) catRAPID predicted binding region of HOTTIP and EZH2. (b) FISH analyzed the localization of EZH2 and HOTTIP in IR-K562 cells. (c) RIP-PCR were used to test the interaction between EZH2 and HOTTIP. \*  $p < 0.05$  vs. IgG. (d) RT-qPCR and Western blot were used to measure EZH2 mRNA and protein level after knocking down HOTTIP. (e) Western blot analysis was used to measure EZH2 in BM-MNCs of CML patients. Right panel, bar chart of protein densitometric analysis. \*\*\*  $p < 0.001$ . RT-qPCR was used to detect EZH2 level in BM-MNCs of CML patients. Normalized to GAPDH. \*\*\*  $p < 0.001$  vs. NC. Western blot analysis was used to measure EZH2 protein level in IR-K562 and K562 cells. Right panel, bar chart of protein densitometric analysis. \*\*\*  $p <$

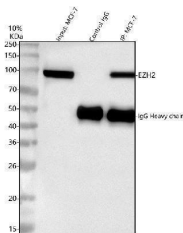
0.001; RT-qPCR was used to detect EZH2 mRNA level in CML cell lines (K562 and IR-K562). Normalized to GAPDH. \*\*\* P < 0.001 vs. NC. (f) RT-qPCR and Western blot were used to detect EZH2 and PTEN mRNA and protein levels after transfecting with specific sh-con or sh-EZH2. \*\*\* P < 0.001 vs. DMSO. Index in PubMed under a CC BY license. PMID: 36117724



HOTTIP work through EZH2 to participate in drug resistance. Note: (a) IR-K562 cells were transfected with specific sh-HOTTIP or both sh-HOTTIP+sh-EZH2. The CCK-8 analysis was used to detect cell proliferation. (b) Cell apoptosis was detected by flow cytometry using Annexin V/APC/PI. The right panel shows the apoptosis rate from three independent experiments. (c) IR-K562 cells were prepared with different Imatinib concentrations for 48 h. The CCK-8 analysis was used to detect cell inhibition. (d) WB was used to detect protein levels. Index in PubMed under a CC BY license. PMID: 36117724



Western blot analysis of EZH2 using anti-EZH2 antibody (A00050-1). Electrophoresis was performed on a 8% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human THP-1-WT whole cell lysates, Lane 2: human THP-1-EZH2 KO whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-EZH2 antigen affinity purified polyclonal antibody (A00050-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for EZH2 at approximately 98 kDa. The expected band size for EZH2 is at 85 kDa.



Immunoprecipitating KMT6/EZH2 in MCF-7 whole cell lysate. Western blot analysis of KMT6/EZH2 using anti-KMT6/EZH2 antibody (A00050-1). Lane 1: MCF-7 whole cell lysates (30ug); Lane 2: Rabbit control IgG instead of anti-KMT6/EZH2 antibody in MCF-7 whole cell lysate; Lane 3: anti-KMT6/EZH2 antibody (2ug) + MCF-7 whole cell lysate (500ug). After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-KMT6/EZH2 antigen affinity purified polyclonal antibody (A00050-1) at a dilution of 0.5 ug/mL and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1196-200). A specific band was detected for KMT6/EZH2 at approximately 98 kDa. The expected band size for KMT6/EZH2 is at 85 kDa.

1. PubMed ID: 26265454, Enhancer of zeste homolog 2 silencing inhibits tumor growth and lung metastasis in osteosarcoma

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Anti-KMT6/EZH2 Antibody

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