

## PRODUCT INFORMATION

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**Product name :** EZH2 antibody

**Product type :** Primary antibodies

**Description :** Rabbit polyclonal to EZH2

**Immunogen :** 3 synthetic peptides (human) conjugated to KLH

**Reacts with :** Human, Mouse

**Tested applications :** ELISA, WB & IF

## GENE INFORMATION

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**Gene Symbol :** EZH2

**Gene Name :** enhancer of zeste homolog 2 (Drosophila)

**Ensembl ID :** ENSG00000106462

**Entrez GeneID :** 2146

**Omim ID :** 601573

**Swiss-Prot :** Q15910

**Molecular weight of EZH2 :** 86, 85.4 & 81kDa

**Function :** Polycomb group (PcG) protein. Catalytic subunit of the PRC2/EED-EZH2 complex, which methylates 'Lys-9' and 'Lys-27' of histone H3, leading to transcriptional repression of the affected target gene. Able to mono-, di- and trimethylate 'Lys-27' of histone H3 to form H3K27me1, H3K27me2 and H3K27me3, respectively. Compared to EZH2-containing complexes, it is more abundant in embryonic stem cells and plays a major role in forming H3K27me3, which is required for embryonic stem cell identity and proper differentiation. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes

**Expected subcellular localization :** Nucleus

**Summary :** 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. [provided by RefSeq, Feb 2011]

## APPLICATION NOTE

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### Recommended dilution :

- ELISA: Antibody specificity was verified by direct ELISA against the 3 immunogen peptides. A minimum titer of 1/20000 is determined. Appropriate specificity controls were run.
- WB (recombinant protein): 1/5000.
- WB (cell line lysate): 1/500.
- IF: 1/500

Optimal dilutions/concentration should be determined by the end user.

**Raised in :** Rabbit

**Clonality :** Polyclonal

**Isotype :** IgG

**Purity :** Purified Antibody

**Storage buffer :** 0.5 X PBS, 50% glycerol containing a final concentration of 0.1% BSA and 0.01% Thimerosal.

**Form :** Liquid

**Storage instruction :** Store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

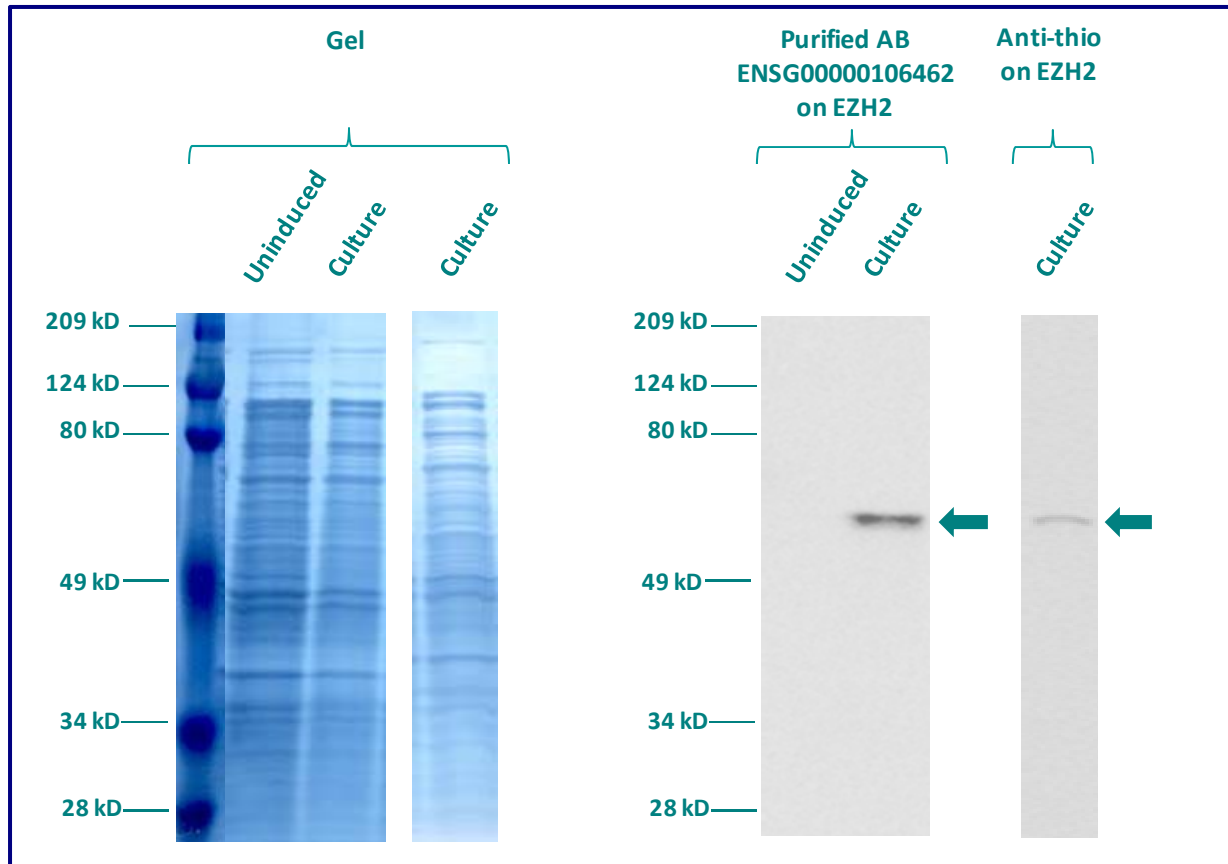
## WESTERN BLOT ON RECOMBINANT PROTEIN

The purified antibody ENSG00000106462 has been tested at 1/25000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

An anti-thio has been tested at 1/5000 on induced culture of E.coli (one shot Top10 competent cells) as a positive control.

Plasmid name : pBAD-DEST49.

Molecular weight of EZH2 : 99.4kDa (85.4kDa + another 14kDa for the tag).



Gel concentration: 10%

Blocking: in 5% non-fat milk-PBST solution

1<sup>st</sup> Antibody: The antibodies are diluted in blocking buffer.

- Dilute the purified antibody ENSG00000106462 at 1: 5000
- Dilute the anti-thio at 1:5000

60 minutes of incubation

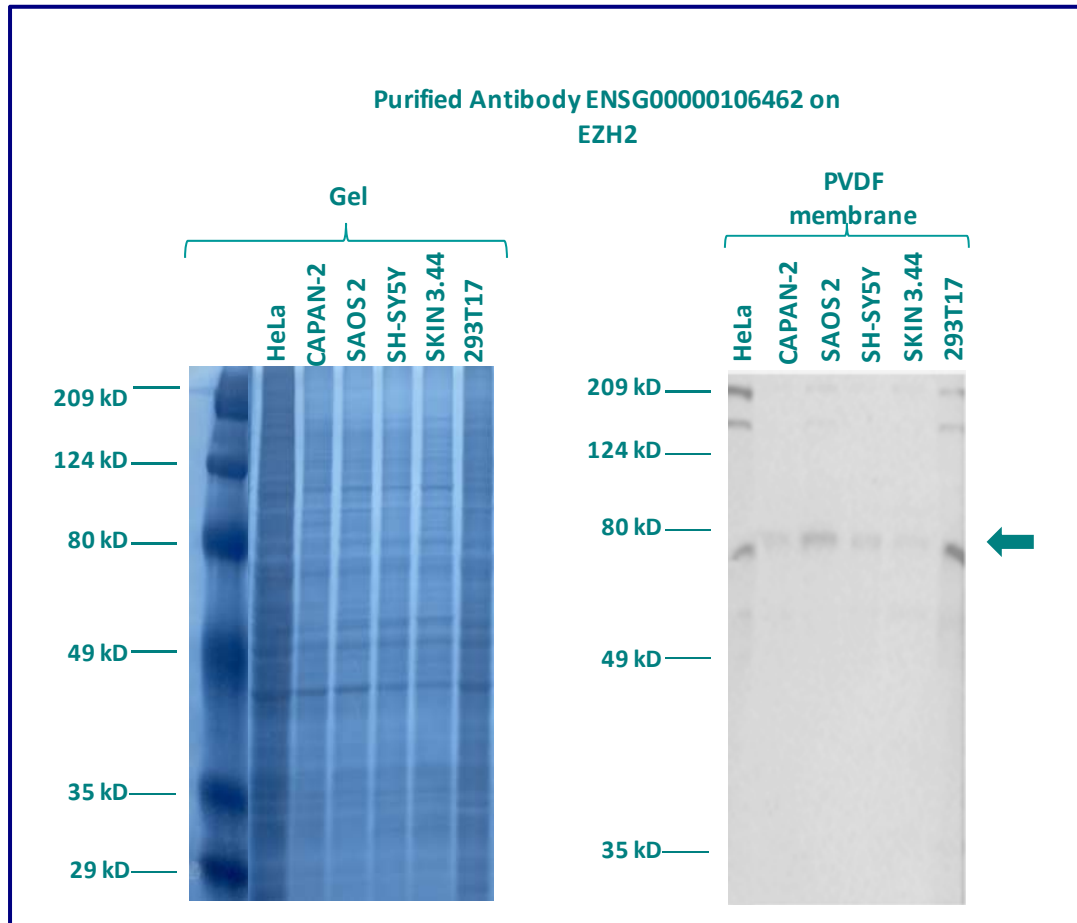
2<sup>nd</sup> Antibody: The antibody is diluted in blocking buffer.

- Dilute the anti-Rabbit IgG HRP conjugated at 1/10000
- 60 minutes of incubation

## WESTERN BLOT ANALYSIS ON CELL LYSATES

The purified antibody ENSG00000106462 has been tested at a concentration of 1/500 on total protein extract of various cell lines (HeLa, CAPAN-2, SAOS 2, SH-SY5Y, SKIN 3.44 & 293T17).

Molecular weight of EZH2 isoforms : 86, 85.4 & 81kDa



**Gel concentration: 10%**

**Blocking: in 5% non-fat milk-PBST solution**

**1<sup>st</sup> Antibody: The antibodies are diluted in blocking buffer.**

- Dilute the purified antibody ENSG00000106462 at 1: 500  
60 minutes of incubation

**2<sup>nd</sup> Antibody: The antibody is diluted in blocking buffer.**

- Dilute the anti-Rabbit IgG HRP conjugated at 1/10000  
60 minutes of incubation

## IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescence analysis of Histone-lysine N-methyltransferase EZH2 (EZH2) expression in 5 cells lines (HELA, 293T/17, Capan-2, SH-SY5Y, Skin 3,44). The purified Antibody ENSG00000106462 has been tested at 1/500.

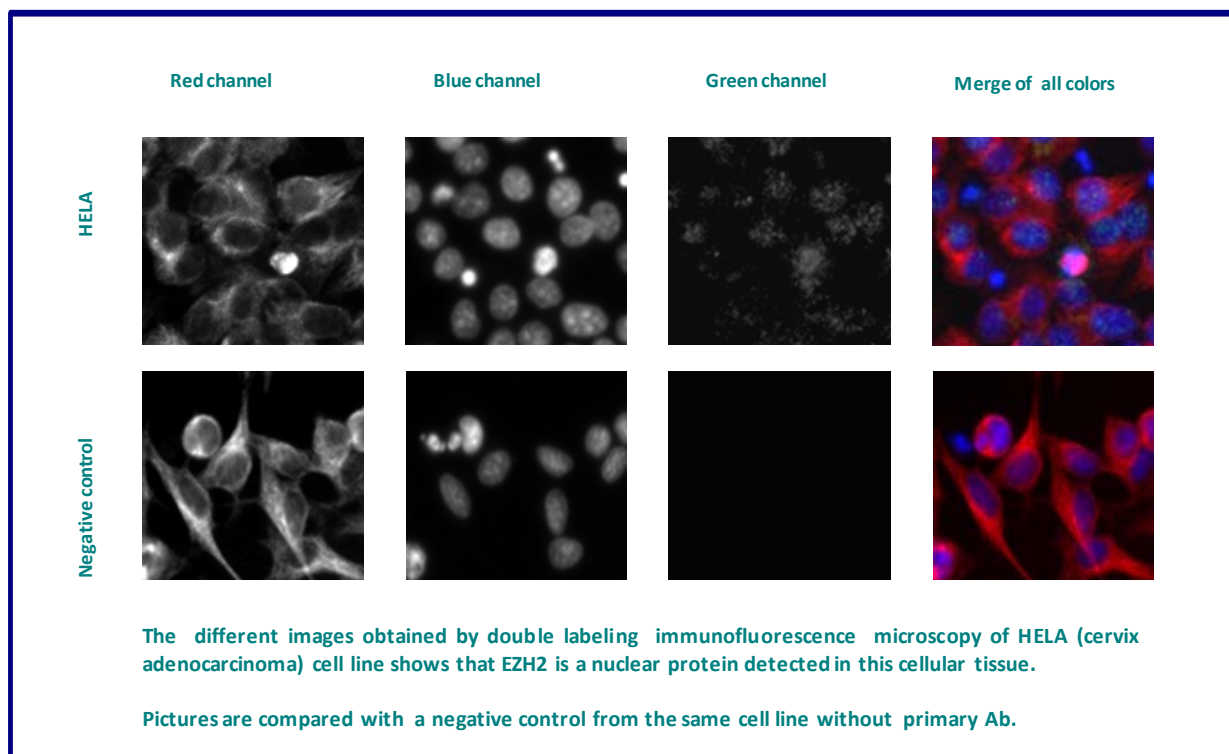
**Red staining** : cytoskeleton (microtubules/ $\alpha$ -tubuline)

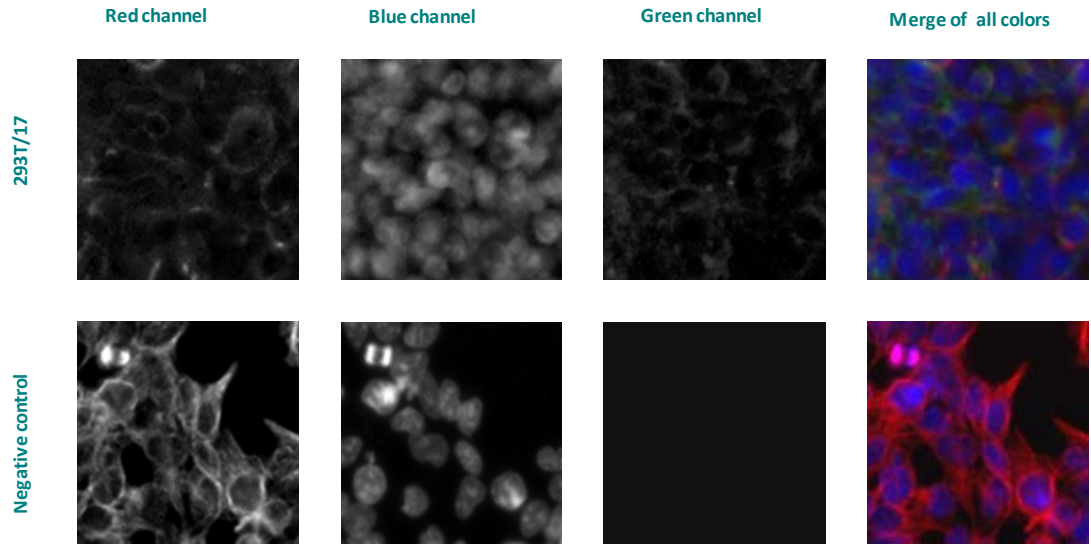
**Blue staining** : nucleus (Hoechst)

**Green staining** : anti- EZH2 antibody (purified)

**Expected subcellular location** : Nucleus

**Expected tissue specificity** : Expressed in many tissues. Overexpressed in numerous tumor types including carcinomas of the breast, colon, larynx, lymphoma and testis.





The different images obtained by double labeling immunofluorescence microscopy of 293T/17 (kidney embryonic) cell line shows that EZH2 is a cytoplasmic protein detected in this cellular tissue.

Pictures are compared with a negative control from the same cell line without primary Ab.

Remaining cell lines tested gave a positive result with a cytoplasmic distribution.