

## Technical Data Sheet

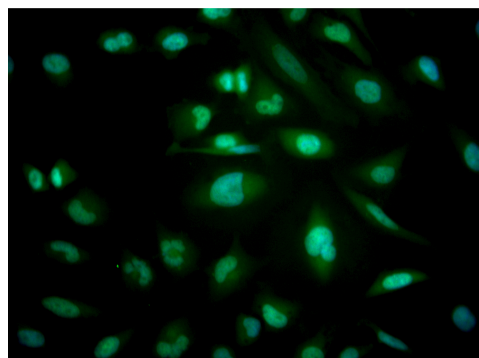
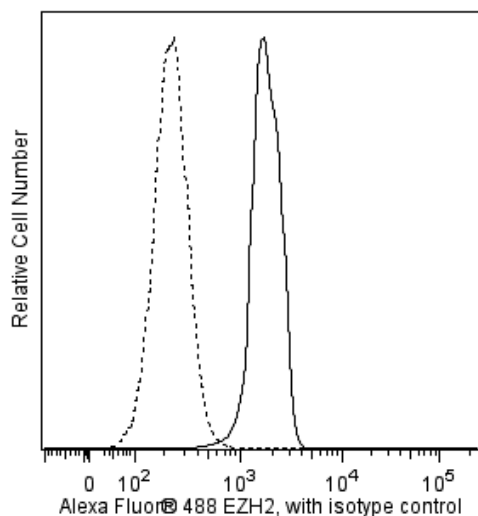
## Alexa Fluor® 488 Mouse anti-EZH2

## Product Information

<b>Material Number:</b>	562479
<b>Alternate Name:</b>	Enhancer of zeste 2; ENX-1; Histone-lysine N-methyltransferase EZH2; KMT6
<b>Entrez Gene ID:</b>	2146
<b>Size:</b>	50 tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	11/EZH2
<b>Immunogen:</b>	Human EZH2 recombinant protein aa. 156-256
<b>Isotype:</b>	Mouse IgG1
<b>Reactivity:</b>	QC Testing: Human Tested by Western blot using purified 11/EZH2 antibody, Cat. No. 612666 or 612667: Chicken, Dog, Mouse, Rat
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

## Description

The 11/EZH2 monoclonal antibody specifically binds to the methyltransferase, EZH2 (Enhancer of Zeste Homolog 2). *EZH2* is a human homologue of *Drosophila's Enhancer of zeste* gene, an important regulator of homeobox gene expression. The EZH2 protein has a predicted molecular weight of ~85 kDa. EZH2 is a member of the Polycomb group (PcG) of proteins that are essential for the maintenance, but not initiation, of the transcriptionally repressed state of certain developmental genes. PcG proteins are a structurally diverse group of proteins with conserved functions from fly to human cells. PcG family proteins form multimeric complexes that regulate the expression of genes involved in cell cycle, DNA repair and differentiation. Specifically, EZH2 is a core enzymatic component of PRC2 (polycomb repressive complex 2). EZH2 is expressed in some lymph node follicular T cells and B cells. Thymocytes differentially express EZH2 at various stages during T-cell maturation. EZH2 interacts with multiple signaling proteins, including Vav, that are involved in lymphocyte development and activation. It is highly expressed in a variety of tumors including lymphomas as well as breast and prostate cancers. EZH2 is important in the self renewal and proliferation of numerous stem cell types including fetal hematopoietic stem cells, muscle satellite cells, hepatic stem/progenitor cells, neural stem cells, basal cell progenitors in the developing epidermis, embryonic stem cells, and some cancer stem cells.



**LEFT: Flow cytometric analysis of EZH2 expression in a human cell line.** Jurkat cells (ATCC, TIB-152™) were harvested, fixed in BD Cytotfix™ Fixation Buffer (Cat. No. 554655), permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) and stained with matching concentrations of either Alexa Fluor® 488 Mouse IgG1, κ Isotype Control (Cat. No. 557721; dashed line histogram) or Alexa Fluor® 488 Mouse anti-EZH2 antibody (Cat. No. 562479; solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable Jurkat cells. Flow cytometry was performed using a BD FACS Canto™ II Flow Cytometer System.

**RIGHT: Immunofluorescent staining and image analysis of EZH2 expression in a human cell line.** HeLa cells (ATCC, CCL-2™) were fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655), permeabilized with 0.1% Triton™-X 100 (Sigma Cat. No. X-100), and stained with Alexa Fluor® 488 Mouse anti-EZH2 monoclonal antibody (Cat. No. 562479; pseudo colored green) at 10 µg/mL. BD Pharmingen™ Hoechst 33342 solution (Cat. No. 561908; pseudo-colored blue) was used for counterstaining. The image was captured using a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software.

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## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

## Application Notes

### Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Tested During Development
Immunofluorescence	Tested During Development

### Recommended Assay Procedure:

- This antibody conjugate is suitable for intracellular staining of human cell lines using BD Cytofix™ Fixation Buffer. BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) and BD Phosflow™ Perm Buffer III (Cat. No. 558050) can be used with this antibody conjugate.
- The Bioimaging protocol can be found at [http://www.bdbiosciences.com/support/resources/protocols/certified\\_reagents.jsp](http://www.bdbiosciences.com/support/resources/protocols/certified_reagents.jsp).

### Suggested Companion Products

Catalog Number	Name	Size	Clone
557721	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
561908	Hoechst 33342 Solution	1.0 mg	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)

### Product Notices

- This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-μl experimental sample (a test).
- This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
- An isotype control should be used at the same concentration as the antibody of interest.
- Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
- The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- Triton is a trademark of the Dow Chemical Company.
- All other brands are trademarks of their respective owners.
- Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

### References

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