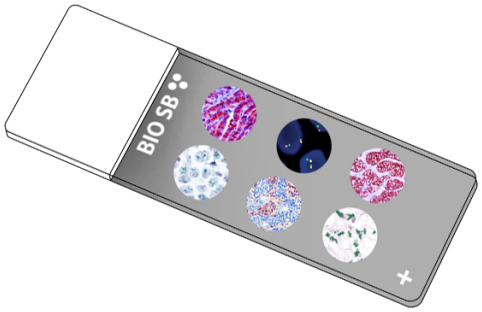
BIO SB
A CANCER DIAGNOSTICS COMPANY

EZH2 Control Slides

RUO



Intended Use
For Research Use Only.

Summary and Explanation
Enhancer of zeste homolog 2 (EZH2) is an enzymatic catalytic subunit of polycomb repressive complex 2 (PRC2), which is involved in trimethylation of Lys 27 on Histone 3 and GATA4. Histone methylation and chromatin regulation are affected by EZH2 dysregulation and can lead to increased cell survival and cancer development as downstream genes are not properly silenced. EZH2 expression in naïve T cells promotes survival, proliferation, and function of effector T cells. EZH2 plays a key role for the function of tumor-specific effector T cells, while tumor microenvironments reduce EZH2 expression of T cells via glucose restriction and in turn, mediates T cell dysfunction.

EZH2 may inhibit apoptosis in ectopic Breast Cancer, overexpression is correlated with poor clinical outcome in Prostate and Gastric Cancer. EZH2 overexpression is positively correlated with epithelial-mesenchymal transition, tumor size, lymphatic invasion and TNM stage, and poor disease-free survival and overall survival of patients. EZH2 regulates ADAR1 expression, and EZH2 and BET inhibitors show synergistic inhibition in pancreatic cancer. EZH2 expression is significantly higher in BAP1-mutant renal clear cell carcinoma patients with progressed stage, grade, nodal invasion, and metastasis.

Presentation
Five slides of EZH2 positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

Catalog No.	*Quantity*
BSB-9447-CS-RUO	5 slides

Storage Store at 20-25°C

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information, refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Stability
This product is stable up to the expiration date on the product label.
Do not use after expiration date listed on package label.

IHC Protocol
1. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
2. Any of three heating methods may be used:
a. TintoRetriever Pressure Cooker or Equivalent
Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.
b. TintoRetriever PT Module or Water Bath Method
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.
c. Conventional Steamer Method
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.
3. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
4. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
5. Wash slides with ImmunoDNA washer or DI water.
6. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate- Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Abbreviated IF Protocol

Step	Incubation Time
Rinse slides in IF wash buffer	5 minutes
Drain and wipe excess IF wash buffer off slide	
Conduct remaining steps in the dark	
Apply Antibody	30-60 minutes
Rinse with 3 changes of IF wash buffer	3x15 minutes each
Coverslip with IF mounting medium	

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized, and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References

1. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. J Hematol Oncol. 2020;13(1):104.
2. Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med. 2016;22(2):128-134.
3. Kang N, et al. EZH2 inhibition: a promising strategy to prevent cancer immune editing. Epigenomics. 2020;12(16):1457-1476
4. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement/Vol. 61, January 6, 2012. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Product Limitations

Symbol Key / Légende des symboles/Erläuterung der Symbole

Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	Expiration Date Utiliser jusque Verwendbar bis	LOT Lot Number Code du lot Chargenbezeichnung

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