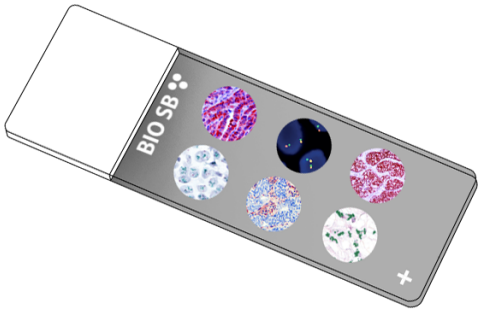
MGMT/AGAT Control Slides

CE IVD



Intended Use
For In Vitro Diagnostic Use.

Summary and Explanation
MGMT is a house-keeping gene expressed in all tissues, and its promoter methylation and resulting down-regulation of the AGT protein varies among tumor types. The MGMT promoter contains many regulatory domains, and methylation can help decrease protein expression and therefore resistance to drugs, improving effectiveness of alkylating treatments.High MGMT expression has been reported in Glioma, Myeloma, Melanoma, Colon and Pancreatic Cancers, where inhibition or down-regulation make alkylating drug treatments more effective. MGMT expression is lost in some cancer types such as Lymphomas, Non-Small Cell Lung Cancer, Astrocyte Tumors, Breast, and Prostate Cancer, where it may fail to prevent mutations.A study detected protein expression of MGMT by IHC and MGMT promoter methylation detected by methylation-specific polymerase chain reaction were performed in a series of newly diagnosed Glioblastomas and found that the MGMT status detected by either IHC or MSP was significantly correlated with the treatment response and survival of Glioblastoma patients. MGMT methylation is frequently observed in sporadic colorectal cancer and was recently correlated with the C>T allele at SNP rs16906252, within the transcriptional enhancer element of the promoter. MGMT methylation has also been associated with KRAS mutations, particularly G>A transitions. Another study address the association between sequence variants within the MGMT (O(6)-methylguanine-DNA methyltransferase) promoter-enhancer region and methylation of MGMT in premalignant lesions from smokers and lung adenocarcinomas, their biological effects on gene regulation, and targeting MGMT for therapy and found strong evidence that the A allele of a MGMT promoter-enhancer SNP is a key determinant for MGMT methylation in lung carcinogenesis. Moreover, TMZ treatment may benefit a subset of lung cancer patients methylated for MGMT.

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

Catalog No.	*Quantity*
BSB-9279-CS	5 slides
BSB-3737-CS	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 the 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.

Product Limitations

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. Lipp ES, Healy P, Austin A, et al. MGMT: Immunohistochemical Detection in High-Grade Astrocytomas. J Neuropathol Exp Neurol. 2019;78(1):57-64. doi:10.1093/jnen/nly110
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3. Sharma S, Salehi F, Scheithauer BW, Rotondo F, Syro LV, Kovacs K. Role of MGMT in tumor development, progression, diagnosis, treatment and prognosis. Anticancer Res. 2009;29(10):3759-3768
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7. Hawkins NJ, Lee JH, Wong JJ, Kwok CT, Ward RL, Hitchins MP. MGMT methylation is associated primarily with the germline C>T SNP (rs16906252) in colorectal cancer and normal colonic mucosa. Mod Pathol. 2009;22(12):1588-1599. doi:10.1038/modpathol.2009.130
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Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REP QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
IVD In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	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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