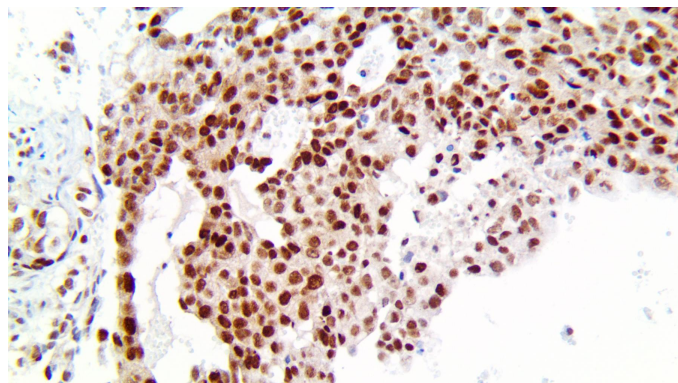


MGMT/AGAT

Clone: EP337
Rabbit Monoclonal



Inset: IHC of MGMT/AGAT on a FFPE Lung Adenocarcinoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections, and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The MGMT antibody, clone EP337, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

A synthetic peptide corresponding to residues of the human MGMT protein.

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 (MSP) 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.

Antibody Type	Rabbit Monoclonal	Clone	EP337
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Nuclear, Cytoplasmic	Species Reactivity	Human
Control	Breast, Cervix, Fallopian Tube, Prostate, Testis, Transitional Cell Carcinoma, Lung Adenocarcinoma, Ductal Breast Carcinoma, Endometrial Carcinoma		
Application	Neural & Neuroendocrine Cancer, Breast Cancer, Leukemia and Histiocytic Cancers, Melanoma and Skin Cancer, Colon and Gastrointestinal Cancer, Gallbladder and Pancreatic Cancer, Lung Cancer, Prostate Cancer, Lymphoma		

Presentation

Anti-MGMT/AGAT is a Rabbit Monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

<i>Catalog No.</i>	<i>Presentation</i>	<i>Dilution</i>	<i>Volume</i>
BSB-3737-3	Predilute	Ready-to-Use	3.0 mL
BSB-3737-7	Predilute	Ready-to-Use	7.0 mL
BSB-3737-15	Predilute	Ready-to-Use	15.0 mL
BSB-3737-01	Concentrate	1:50-1:200	0.1 mL
BSB-3737-05	Concentrate	1:50-1:200	0.5 mL
BSB-3737-1	Concentrate	1:50-1:200	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB-3737-CS	5 slides

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN₃) as a preservative. 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. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. 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. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033), or ImmunoDNA Digester (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used on acetone-fixed frozen sections and acetone-fixed cell preparations.

IHC Protocol

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. 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).
5. 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.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

Symbol Key / Légende des symboles/Erläuterung der Symbole

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
	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 Number Code du lot Chargenbezeichnung

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

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

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