CANAL CONTRACT

MLH1 Clone: G168-728 Mouse Monoclonal



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Inset: IHC of MLH1 on a Colon Carcinoma Tissue

### **Intended** Use

For In Vitro Diagnostic Use.

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

#### Immunogen

Full length recombinant MLH1 protein.

### **Summary and Explanation**

MLH1 is a mismatch repair gene of around 87 kDa, commonly associated with Hereditary Non-Polyposis Colorectal Cancer (HNPCC). This gene was identified as a locus frequently mutated in HNPCC. It is a human homolog of the E. coli DNA mismatch repair gene mutL, consistent with the characteristic alterations in microsatellite sequences (RER+ phenotype) found in HNPCC. Alternatively, spliced transcript variants encoding different isoforms have been described, but their fulllength natures have not been determined.

In a high proportion of patients with microsatellite instability (MSI-H), the MLH1 protein is typically deficient. This protein deficiency is linked to the autosomal dominant condition of Hereditary Non-Polyposis Colon Cancer. The anti-MLH1antibody is useful in screening patients and families for this condition. Colon cancers that are microsatellite-unstable have a better prognosis than their microsatellite stable counterparts.

Antibody Type	Mouse Monoclonal	Clone	G168-728		
lsotype	lgG2a/K	Reactivity	Paraffin, Frozen		
Localization	Nuclear	Control	Testis, Tonsil, Colon, Kidney, Colon Carcinoma		
	Species Reactivity	Human, Rat, Mouse			

### Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.

2. This product contains <0.1% sodium azide (NaN<sub>3</sub>) as a preservative. Ensure proper handling procedures are used with this reagent.

3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.

4. Dispose of unused solution with copious amount of water.

Diagnostic Laboratories" (see References in this document).

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).

 For additional safety information refer to Safety Data Sheet for this product.
For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical

### Presentation

Anti-MLH1 is a mouse 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.

Catalog No.	Antibody Type	Dilution	Volume/Qty	
BSB 5764	Tinto Prediluted	Ready-to-Use	3.0 mL	
BSB 5765	Tinto Prediluted	Ready-to-Use	7.0 mL	
BSB 5766	Tinto Prediluted	Ready-to-Use	15.0 mL	
BSB 5767	Concentrated	1:10 - 1:50	0.1 mL	
BSB 5768	Concentrated	1:10 - 1:50	0.5 mL	
BSB 5769	Concentrated	1:10 - 1:50	1.0 mL	

#### **Control Slides Available**

Catalog No.	Quantity		
BSB 5770	5 slides		

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

### Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on 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 Digestor (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 for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

## **Staining Procedure**

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 staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.

8. Wash slides with ImmunoDNA washer or DI water.

9. 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	

## **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. Wright CL, et al. Am J Surg Pathol. 2003;27:1393-1406
- 2. Brueckl WM, et al. Anticancer Research. 2003;23:1773-1778
- 3. Rigau V, et al. Arch Pathol Lab Med. 2003;127;June:694-700
- 4. Renkonen E, et al. J Clin Oncol. 2003;21:3629-3637
- 5. Hoedema R, et al. The American Surgeon. 2003;May69(5):387-92
- 6. 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.

# Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REP	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	270 Arc	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
	In Vitro Diagnostic Medical Device ssitif médical de diagnostic in vitro In-Vitro-Diagnostikum	[]i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	$\sum$	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung



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