

Inset: IHC of c-Met on a FFPE Papillary Thyroid 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.

* The c-Met antibody, clone EP1454Y, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

Synthetic peptide corresponding to residues near the C-terminus of human Met (c-Met).

Summary and Explanation

c-Met is a proto-oncogene that encodes hepatocyte growth factor receptor (HGFR). The HGFR protein possesses tyrosinase-kinase activity. MET is a membrane receptor that is essential for embryonic development and wound healing, with its only known ligand being hepatocyte growth factor (HGF). Met is normally expressed by cells of epithelial origin, while expression of HGF is restricted to cells of mesenchymal origin. Upon HGF stimulation, MET induces several biological responses that collectively give rise to a program known as invasive growth.

MET is deregulated in many types of human malignancies, including cancers of kidney, liver, stomach, breast, and brain. Normally, only stem cells and progenitor cells express MET, which allows these cells to grow invasively in order to generate new tissues in an embryo or regenerate damaged tissues in an adult. However, cancer stem cells are thought to hijack the ability to express MET, and thus become the cause of cancer persistence and spread to other sites in the body (metastasis).

Antibody Type	Rabbit Monoclonal	Clone	EP1454Y
lsotype	lgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic, Membranous	Control	Breast, Tonsil, Cervix, Papillary Thyroid Carci- noma, Colon Carcinoma
Species Reactivity Hu		Human	

Presentation

C-Met 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.

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Catalog Num.	Antibody Type	Dilution	Volume/Qty	
BSB 6583	Tinto Prediluted	Ready-to-Use	3.0 mL	
BSB 6584	Tinto Prediluted	Ready-to-Use	7.0 mL	
BSB 6585	Tinto Prediluted	Ready-to-Use	15.0 mL	
BSB 6586	Concentrated	1:10 - 1:50	0.1 mL	
BSB 6587	Concentrated	1:10 - 1:50	0.5 mL	
BSB 6588	Concentrated	1:10 - 1:50	1.0 mL	
BSB 6589	Control Slides	Not Applicable	5 slides	

Precautions

Presentations

For professional users only. Ensure results are interpreted by a medical professional.
This product contains sodium azide (NaN3), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.

 Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
Unused solution should be disposed of according to local and federal regulations.
Do not ingest reagent. If reagent ingested, contact a poison control center immediately.

6. 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" (5).

Storage

Store at 2-8 °C. 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 to ensure 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 positive 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 epitope retrieval 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 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 IHC wash buffer or DI water.

9. Continue IHC staining protocol.

Recommended IHC 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	Varies	Varies	Varies	

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 medical professional.

References

- 1. Bottaro D, et al. Science. 1991 Feb; 251(4995):802-4
- 2. Gatland F, et al. Cytogenet. Cell Genet. 1992; 60(2):114-6
- 3. Cooper C, et al. Oncogene. 1992 Jan; 7(1):3-7
- 4. Gentile A, et al. Cancer Metastasis. 2008 March; 27(1):85-94

5. 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 EC	re d	Storage Temperature Limites de température Zulässiger Temperaturbereich	••••	Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
In Vitro Diagnostic Medical Device IVD Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	[]i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	2	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung

Bio SB ??



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