

Inset: IHC of Arginase-1 on a FFPE Hepatocellular 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 Arginase-1 antibody, clone EP261, 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 of human ARG-1 protein.

Summary and Explanation

Arginase-1 is the catalyst for the fifth and final step in the urea cycle, which is a series of biochemical reactions in mammals during which the body disposes of harmful ammonia. Arginase-1 works to convert L-arginine into L-ornithine and urea. Arginise-1 is located primarily in the cytoplasm of the liver. Arginase-1 consists of three tetramers, and the enzyme requires a two-molecule metal cluster of manganese in order to maintain proper function. These Mn2+ ions coordinate with water, orienting and stabilizing the molecule and allowing water to act as a nucelophile and attack L-arginine, hydrolyzing it into ornithine and urea.

Arginase-1 is abundantly expressed in the liver and it represents a sensitive and specific marker of benign and malignant hepatocytes. In sections of normal liver, anti-arginase 1 produces strong, diffuse cytoplasmic reactivity in all hepatocytes throughout the lobule. In a small percentage of cases, patchy nuclear reactivity is also evident in hepatocytes along with the strong cytoplasmic reactivity. Hepatocellular carcinoma usually shows higher protein expression of ARG1 than normal liver cells.

Antibody Type	Rabbit Monoclonal	Clone	EP261	
lsotype	lgG	Reactivity	Paraffin, Frozen	
Localization	Cytoplasmic, Nuclear	Control	Liver, Hepatocellular Carcinoma	
Species Reactivity		Human		

Presentation

Arginase-1 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.

Catalog Num.	Antibody Type	Dilution	Volume/Qty
BSB 2447	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2448	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2449	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2450	Concentrated	1:50 - 1:200	0.1 mL
BSB 2451	Concentrated	1:50 - 1:200	0.5 mL
BSB 2452	Concentrated	1:50 - 1:200	1.0 mL
BSB 2453	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.

3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
4. Unused solution should be disposed of according to local and federal regulations.
5. 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 copin 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 PolyDetector AP/HRP 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	

Performance Characteristics

Normal Tissues					
Positive (+)					
Liver	10/10 (100%)				
Negative (-)					
Kidney	0/5 (0%)				
Testis	0/5 (0%)				
Tonsil	0/5 (0%)				
Spleen	0/5 (0%)				
Abnormal Tissues					
Positive (+)					
Hepatocellular Carcinoma	7/9 (77%)				
Hepatic Adenoma	9/12 (75%)				
Negative (-)					
Metastatic Adenocarcinoma	0/15 (0%)				
Lymphoblastic Lymphoma	0/11 (0%)				
Bladder TCC	0/15 (0%)				
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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. Wu G, et al. Biochemical Journal. 1998 Nov.; 336(1):1-17
- 2. Di Costanzo L, et al. Archives of biochemistry and biophysics. 2007 Sept.; 465(1):82-9
- 3. Multhaupt H, et al. Histochemistry. 1987; 87:465-70
- 4. Sekine S, et al. J Pathol. 2009: 219:365-72

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	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	2°C	Storage Temperature Limites de température Zulässiger Temperaturbereich	***	Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
	tro Diagnostic Medical Device médical de diagnostic in vitro In-Vitro-Diagnostikum	Ĩ	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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