

Inset: IHC of SOX10 on a FFPE Melanoma 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 SOX10 antibody, clone EP268, has been manufactured using Epitomics RabMab[®] technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

A recombinant fragment corresponding to residues in human SOX10 protein.

Summary and Explanation

Transcription factor SOX-10 is a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and in the determination of the cell fate. The encoded protein may act as a transcriptional activator after forming a protein complex with other proteins. This protein acts as a nucleocytoplasmic shuttle protein and is important for neural crest and peripheral nervous system development. Mutations in this gene are associated with Waardenburg-Shah and Waardenburg-Hirschsprung disease. Anti-SOX-10 has been recently shown to be a sensitive marker of melanoma, including conventional, spindled, and desmoplastic subtypes.

SOX-10 is expressed by metastatic melanomas and nodal capsular nevus in sentinel lymph nodes, but not by other lymph node components such as dendritic cells which usually express S100 protein. In scar specimens, immature fibroblasts, epithelioid granulomas, and histiocytic proliferations can histopathologically mimic residual melanoma and even be positive for MiTF and S100. However, SOX-10 is less likely to be expressed by fibroblasts or histiocytes, especially compared to MiTF and S100. Anti-SOX-10 produces a nuclear stain that provides a clean signal that is much sharper and darker in staining quality when compared to the use of antibodies against MiTF and S100.

Antibody Type	Rabbit Monoclonal	Clone	EP268	
lsotype	lgG	Reactivity	Paraffin, Frozen	
Localization	Nuclear	Control	Skin, Melanoma, Schwanoma	
Species Reactivity		Human, Predicted: Mouse, Rat		

Presentation

SOX10 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 2580	Tinto Prediluted	Ready-to-Use	3.0 mL		
BSB 2581	Tinto Prediluted	Ready-to-Use	7.0 mL		
BSB 2582	Tinto Prediluted	Ready-to-Use	15.0 mL		
BSB 2583	Concentrated	1:50 - 1:200	0.1 mL		
BSB 2584	Concentrated	1:50 - 1:200	0.5 mL		
BSB 2585	Concentrated	1:50 - 1:200	1.0 mL		
BSB 2586	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 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	

Performance Characteristics

Normal Tissues					
Positive (+)					
Breast 4/5 (80%)					
Nevus	10/10 (100%)				
Negative (-)					
Pancreas	0/5 (0%)				
Testis	0/5 (0%)				
Thyroid	0/5 (0%)				
Tonsil	0/5 (0%)				
Abnormal Tissues					
Posit	ive (+)				
Metastatic Melanoma	5/6 (83%)				
Schwanoma	2/2 (100%)				
Spindle Cell Melanoma	7/7 (100%)				
Negative (-)					
Bladder TCC	0/5 (0%)				
Lymphoblastic Lymphoma	0/5 (0%)				

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. Pingault V, et al. Nat Genet. 1998 Feb; 18(2):171-3
- 2. Bondurand N, et al. Hum Mol Genet. 2000 Jan; 8(9):1785-9
- 3. Chorny JA, et al. Am J Dermatopathol. 2002; 24:309
- 4. Robson A, et al. Histopathology. 2001; 38:135

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 EXERCISE 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
In Vitro Diagnostic Medical Device Dispositif 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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