

Inset: IHC of Stathmin on a FFPE Anal Carcinoma Tissue

Drocontations

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 Stathmin antibody, clone EP247, 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 the stathmin protein

Summary and Explanation

Stathmin 1/oncoprotein 18, also known as STMN1, is a highly conserved 17 kDa protein. Stathmin performs an important function in regulating rapid microtubule remodeling of the cytoskeleton in response to the cell's needs. Regulation of stathmin is cell cycle dependent and controlled by the cell's protein kinases in response to specific cell signals. Stathmin can cause uncontrolled cell proliferation when mutated and not functioning properly.

Overexpression of Stathmin has been associated with tumor progression in endometrial carcinomas, ovarian cancer and oral squamous-cell carcinoma . Stathmin has been found to be positive in 29% of CINs with differential expression based on the grade of the lesion as 9% being CIN1, 45% CIN2, and 93% CIN3; whereas, p16 staining of the same cases was positive in 80% of CINs with 71% CIN1, 100% CIN2, and 94% CIN3. Stathmin shows similar sensitivity for CIN3 to anti-p16 (93% vs 94%) although it drops off for CIN2 (73% vs 96%). The specificity of anit-stathmin for both CIN2-3 (94%) and CIN3 (89%) is higher than that of anti-p16 (44% and 39%, respectively). Therefore, Stathmin has major potential as a diagnostic marker in CIN classification over anti-p16 and it is valuable to distinguish CIN3 from the majority of low-grade precursors and negative/reactive cervical biopsies.

Antibody Type	Rabbit Monoclonal	Clone	EP247		
lsotype	IgG	Reactivity	Paraffin, Frozen		
Localization Cytoplasmic		Control	Testis, Tonsil, HSIL Cervical Carcinoma, Lym- phoblastic Lymphoma, Bladder TCC		
Species Reactivity		Human, Predicted: Mouse, Rat			

Presentation

Stathmin 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 2594	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2595	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2596	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2597	Concentrated	1:50 - 1:200	0.1 mL
BSB 2598	Concentrated	1:50 - 1:200	0.5 mL
BSB 2599	Concentrated	1:50 - 1:200	1.0 mL
BSB 2600	Control Slides	Not Applicable	5 slides

Precautions

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" (8).

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 (+)						
Testis	5/5 (100%)					
Tonsil	5/5 (100%)					
Nega	tive (-)					
Lung	0/5 (0%)					
Heart	0/5 (0%)					
Abnormal Tissues						
Positi	ive (+)					
HSIL	20/20 (100%)					
Lymphoblastic Lymphoma	6/6 (100%)					
Melanoma	15/16 (93%)					

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. Atweh, G., et al. J Cellular Biochemistry 2004; 93 (2): 242–250.
- 2. Cajone F, et al.: Clin Exp Metastasis 1999, 17:865-871
- 3. Curmi PA, et al.: Br J Cancer 2000, 82:142-150
- 4. Price DK, et al.: Cancer Invest 2000, 18:722-730
- 5. Trovik J, et al.: Clin Cancer Res 2011, 17:3368-3377
- 6. Su D, et al.: Cancer 2009, 115:2453-2463
- 7. Howitt B, et al.: Am J Surg Pathol 2013, 37:89-97

8. 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
IVD Disp	In Vitro Diagnostic Medical Device ositif 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





69 Santa Felicia Dr., Santa Barbara, CA 93117, USA Tel: (805) 692-2768 | Tel: (800) 561-1145 | Fax: (805) 692-2769 E-mail: info@biosb.com | Website: www.biosb.com