



Inset: IHC of Annexin A1 on a FFPE Hairy Cell Leukemia Tissue

Intended Use

For In Vitro Diagnostic Use.

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

Immunogen

Human Annexin A1.

Summary and Explanation

The protein Annexin A1 is encoded by the ANXA1 gene, which is upregulated in hairy cell leukemia. The NFkB signal transduction pathway is exploited by cancerous cells to proliferate and avoid apoptosis. Annexin A1 inhibits that pathway by binding to the p65 subunit, thus making Annexin A1 of particular interest for use as a potential anti-cancer drug. It may also contain tumor suppressive and protective characteristics, as has been evidenced by its ability to protect against DNA damage induced by heat in breast cancer cells.

Annexin A1 is strongly expressed on the cell membrane and occasionally in the cytoplasm of tumor cells in 97% of samples from patients with hairy cell leukemia. By contrast, B-cell lymphomas other than hairy cell leukemia are ANXA1 negative. Thus, ANXA1 is a molecule specific to hairy cell leukemia that can be used to differentiate this disease from other B-cell lymphomas.

Antibody Type	Mouse Monoclonal	Clone	BSB-95
Isotype	lgG2b	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic, Membranous	Control	Liver, Tonsil, Spleen, Thymus, Lung, Colon, Hairy Cell Leukemia
Species Reactivity		Human, Dog	

Presentation

Anti-Annexin A1 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.

Presentations

Catalog Num.	Antibody Type	Dilution	Volume/Qty
BSB 6359	Tinto Prediluted	Prediluted Ready-to-Use	
BSB 6360	Tinto Prediluted	Tinto Prediluted Ready-to-Use	
BSB 6361	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 6362	Concentrated	1:100 - 1:500	0.1 mL
BSB 6363	Concentrated	1:100 - 1:500	0.5 mL
BSB 6364	Concentrated	1:100 - 1:500	1.0 mL
BSB 6365	Control Slides	Not Applicable	5 slides

Precautions

- 1. For professional users only. Ensure results are interpreted by a medical professional.
- **2.** 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 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 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	

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. Wallner BP, et al. Nature. 1986:320 (6057):77-81
- 2. Zhang Z, et al. Cancer Res. 2010 March; 70 (6):2379-88
- 3. Falini B, et al. Lancet. 2004 June 5; 363 (9424):1869-70
- 4. Xia SH, et al. Oncogene. 2002 Sept. 26; 21(43):6641-8
- 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 RE	P Prinsessegracht 20 2514 AP The Hague The Netherlands	II ~8°C	Storage Temperature Limites de température Zulässiger Temperaturbereich	**	Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
IVD	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	(i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	Σ	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung



