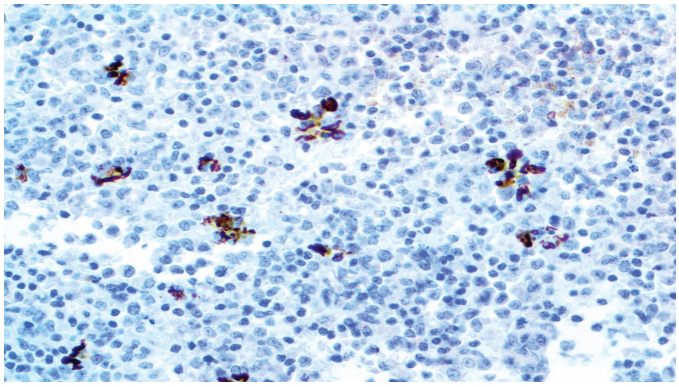
Perforin

Clone: 5B10
Mouse Monoclonal

CE IVD



Inset: IHC of Perforin on a FFPE Lymphoma 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
Recombinant protein corresponding to C-terminal region of human perforin.

Summary and Explanation
Perforin is a cytolytic protein found in the granules of Cytotoxic T lymphocytes and NK cells. Upon degranulation, perforin inserts itself into the target cell's plasma membrane, forming a pore. It enables granzymes to enter the target cells and activate apoptosis, the cell death program. Although some investigators report a cytolytic potential of CD4+ T cells, it appears more likely that CD8+ T cells are the major effector population in Th1- associated inflammatory skin diseases. The role of perforin-mediated cytotoxicity has been demonstrated in various autoimmune diseases. In vitro and in vivo studies suggest that the cytotoxicity of CTLs may be mediated by cytotoxic granules in certain inflammatory diseases in humans. In addition, it seems that T-cell cytotoxicity against keratinocytes is mediated by perforin in some inflammatory skin diseases.

Other authors suggest that perforin may have a dual role in alloimmune response (organ transplant applications). In one regard, it has a cytolytic function in acute rejection, and, in contrast, it may be responsible for downregulating both CD4- and CD8-mediated alloimmune response.

Antibody Type	Mouse Monoclonal	**Clone**	5B10
Isotype	IgG1	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic, Perinuclear	**Species Reactivity**	Human
Control	Spleen		
Application	Rejection & Autoimmunity		

Presentation
Anti-Perforin 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.

Catalog No.	*Presentation*	*Dilution*	*Volume*
BSB 2105	Predilute	Ready-to-Use	3.0 mL
BSB 2106	Predilute	Ready-to-Use	7.0 mL
BSB 2107	Predilute	Ready-to-Use	15.0 mL
BSB 2108	Concentrate	1:50-1:200	0.1 mL
BSB 2109	Concentrate	1:50-1:200	0.5 mL
BSB 2110	Concentrate	1:50-1:200	1.0 mL

Control Slides Available

Catalog No.	*Quantity*
BSB-9343-CS	5 slides

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Precautions
1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. 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" (see References in this document).

Stability
This product is stable up to the expiration date on the product label. 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 for 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 on acetone-fixed frozen sections and acetone-fixed cell preparations.

IHC Protocol

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively 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 induced epitope retrieval (HIER) 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 on trivet 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 IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical 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/Coverslip	Varies	Varies	Varies

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

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 qualified medical professional.

References

1. Tschopp J, et al. Nature. 1986; 322(6082):831-4
2. Chu PG, et al. Ann Diagn Pathol. 1999 April; 3(2):104-33
3. Bittmann I, et al. Virchows Arch. 2004 Oct; 445(4):375-81
4. d'Amore ES, et al. Pediatr Dev Pathol. 2007 May-June; 10(3):181-91
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.
https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Symbol Key/Légende des symboles/Erläuterung der Symbole

EC REP — QAdvis EAR AB, Ideon Science Park, Scheelevägen 17, SE-223 70 Lund, Sweden	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	Read Instructions for Use / Consulter les instructions d'utilisation / Gebrauchsanweisung beachten	Expiration Date / Utiliser jusque / Verwendbar bis	LOT — Lot Number / Code du lot / Chargenbezeichnung

Bio SB
BIOSCIENCE FOR THE WORLD
5385 Hollister Avenue, Bldg. 8, Ste. 108 Santa Barbara, CA 93111, USA
Tel. (805) 692-2768 | Tel. (800) 561-1145 | Fax. (805) 692-2769
E-mail: sales@biosb.com | Website: www.biosb.com