

Inset: IHC of PD-1 on a FFPE Tonsil 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.

#### Immunogen

TY cells (human T/NK cell Leukemia).

## Summary and Explanation

Programmed Death 1 (PD-1 or CD279) is a Type I membrane protein comprised of 268 amino acids. PD-1 is a member of the extended CD28/CTLA-4 family of T-cell regulators. PD-1 is expressed on the surface of activated T-cells, B-cells, and macro-phages. In comparison to CTLA-4, PD-1 more broadly negatively regulates immune responses.

New data suggests that expression of PD-L1 on tumor cells inhibits anti-tumor activity through engagement of PD-1 on effector T-cells. Expression of PD-L1 on tumors is correlated with reduced survival in esophageal, pancreatic and other types of cancers, highlighting the relevance of exploring the PD-1 pathway as a target for immunotherapy. Studies have found that PD-1 is expressed on most T-cells and a small subset of B-cells in the light zone of germinal centers, but not elsewhere in the tonsil. On that basis, it was postulated that PD-1 may play a role in the process of clonal selection of centrocytes, which occurs in this subanatomic site in germinal centers. PD-1 is a new marker of Angioimmunoblastic Lymphoma and suggests a unique cell of origin for this neoplasm. Unlike CD10 and bcl-6, PD-1 is expressed by few B-cells, so it may be a more specific and useful diagnostic marker in Angioimmunoblastic Lymphoma. It also seems to stain a greater percentage of CD3-positive neoplastic cells in Angioimmunoblastic Lymphoma than either CD10 or bcl-6.

Antibody Type	Mouse Monoclonal	Clone	NAT-105		
lsotype	lgG1	Reactivity	Paraffin, Frozen		
Localization	Cytoplasmic, Membranous	Control	Tonsil, Lymph Node, Thymus, Spleen		
Species Reactivity		Human			

## Presentation

PD-1 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 Num.	Antibody Type	Dilution	Volume/Qty
BSB 6212	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 6213	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 6214	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 6215	Concentrated	1:100 - 1:500	0.1 mL
BSB 6216	Concentrated	1:100 - 1:500	0.5 mL
BSB 6217	Concentrated	1:100 - 1:500	1.0 mL
BSB 6218	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 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 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	

# **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. Ishida, et al. Embo. 1992;11:3887
- 2. Agate, et al. Int Immunol. 1996;8:765
- 3. Dorfman DM, et al. Am J Surg Pathol. 2006;30(7):802-10
- 4. Iwai Y, et al. Immunol Lett. 2002;1:83(3):215-20

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	re	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
In Vitro Diagnostic Medical Device <b>IVD</b> Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	[]i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	$\mathbf{x}$	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung

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