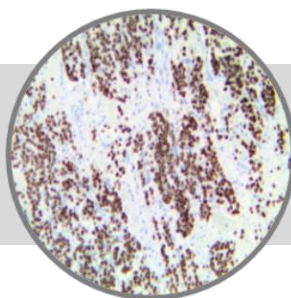


**PHOX2B, RMab****Clone: EP312****Rabbit Monoclonal****IVD****Bio SB**  
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*Inset: IHC of PHOX2B on a FFPE Neuroblastoma Tissue***Intended Use**

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.

\* The PHOX2B antibody, clone EP312, 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 PHOX2B protein.

**Summary and Explanation**

Paired-like homeobox 2b (PHOX2B), also known as neuroblastoma Phox (NBPhox), is a protein that in humans is encoded by the *PHOX2B* gene located on chromosome 4. It is expressed exclusively in the nervous system, in most neurons that control the viscera (cardiovascular, digestive and respiratory systems). It is also required for neuron differentiation. Mutations in human PHOX2B cause a rare disease of the autonomic nervous system (dysautonomia): congenital central hypoventilation syndrome (associated with respiratory arrests during sleep and, occasionally, wakefulness), Hirschsprung's disease (partial agenesis of the enteric nervous system), ROHHAD, and tumors of the sympathetic ganglia.

*PHOX2* gene over-expression in Neuroblastoma (NB) tumors and cell lines suggests these genes may be widely involved in Neuroblastoma development through either a direct mechanism of up-regulation or a failure in maintaining proper transcript levels after embryonic development. The PHOX2B expression has been observed in all peripheral neuroblastic tumors, paragangliomas, and pheochromocytomas tested but in no other pediatric tumors among the 388 cases studied by expression microarray and the 109 cases studied by immunohistochemical analysis. PHOX2B and CD57 have been found to be useful markers of Neuroblastoma. PHOX2B is specific for Neuroblastoma in its differential diagnosis with other small round cell tumors, and its nuclear staining may be helpful for accurate bone marrow tumor quantification.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	EP312
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Nuclear	<b>Control</b>	Adrenal, Neuroblastoma
<b>Species Reactivity</b>		Human, Mouse	

**Presentation**

PHOX2B 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.

<b>Catalog No.</b>	<b>Antibody Type</b>	<b>Dilution</b>	<b>Volume/Qty</b>
BSB 3609	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3610	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3611	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3612	Concentrated	1:25 - 1:100	0.1 mL
BSB 3613	Concentrated	1:25 - 1:100	0.5 mL
BSB 3614	Concentrated	1:25 - 1:100	1.0 mL

**Control Slides Available**

<b>Catalog No.</b>	<b>Quantity</b>
BSB 3615	5 slides

**Precautions**

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN<sub>3</sub>) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount 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).

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

**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 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 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 staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining 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. Longo L, et al. PHOX2A and PHOX2B genes are highly co-expressed in human neuroblastoma. *Int J Oncol.* 2008 Nov;33(5):985-91.
2. Bielle, F, et al. PHOX2B Immunolabeling: A Novel Tool for the Diagnosis of Undifferentiated Neuroblastomas Among Childhood Small Round Blue-cell Tumors. *Am J Surgical Pathol* 2012; (36) 8: 1141-1149.
3. Hata JL, et al. Diagnostic utility of PHOX2B in primary and treated neuroblastoma and in neuroblastoma metastatic to the bone marrow. *Arch Pathol Lab Med.* 2015 Apr;139(4):543-6.
4. 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

		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller		Catalog Number Référence du catalogue Bestellnummer
		Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten				
		Expiration Date Utiliser jusque Verwendbar bis		Lot Number Code du lot Chargenbezeichnung		