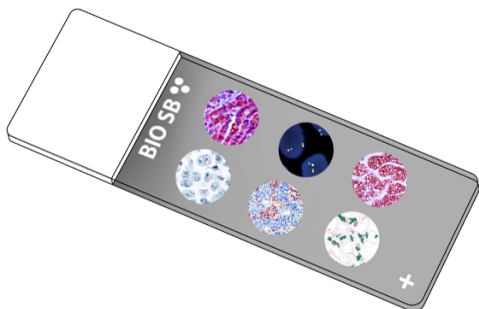


PHOX2B

Control Slides



Intended Use

For In Vitro Diagnostic Use.

Summary and Explanation

Paired-like homeobox 2b (PHOX2B), also known as neuroblastoma Phox, 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 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.

Presentation

Five slides of PHOX2B positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

<i>Catalog No.</i>	<i>Quantity</i>
BSB-9345-CS	5 slides
BSB 3615	5 slides

Storage Store at 20-25°C

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. 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. 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.

IHC Protocol

1. 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).

2. 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.

3. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
4. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
5. Wash slides with ImmunoDNA washer or DI water.
6. 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

Abbreviated IF Protocol

Step	Incubation Time
Rinse slides in IF wash buffer	5 minutes
Drain and wipe excess IF wash buffer off slide	
Conduct remaining steps in the dark	
Apply Antibody	30-60 minutes
Rinse with 3 changes of IF wash buffer	3x15 minutes each
Coverslip with IF mounting medium	

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMunter (BSB 0169-0174) or organic solvent based resin such as PermaMunter (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. <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 jusqu'à Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung