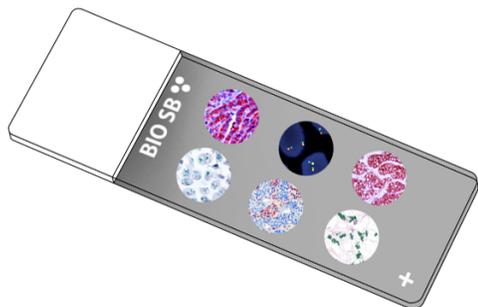


Brachyury Control Slides



Intended Use

For In Vitro Diagnostic Use.

Summary and Explanation

Brachyury is a protein that in humans is encoded by the T gene. Brachyury is a transcription factor within the T-box complex of genes which appears to have a conserved role in defining the midline of a bilaterian organism and thus the establishment of the anterior-posterior axis; this function is apparent in chordates and molluscs. The number of cervical vertebrae is highly conserved among all mammals; however a spontaneous vertebral and spinal dysplasia mutation in this gene has been associated with the development of six or fewer cervical vertebrae instead of the usual seven.

Expression of the brachyury gene has been identified as a definitive diagnostic marker of chordoma, a malignant tumor that arises from remnant notochordal cells lodged in the vertebrae. Furthermore, germ line duplication of brachyury confers major susceptibility to chordoma. The chromosomal region on 6q27 containing the brachyury gene was gained in 6 of 21 chordomas (29%), and none of the 21 chordomas analyzed showed deletions that could have affected this gene.

Brachyury is an important factor in promoting the epithelial-mesenchymal transition. Cells that over-express brachyury have down-regulated expression of the adhesion molecule E-cadherin, which allows them to undergo epithelial-mesenchymal transition. Overexpression of brachyury has been linked to Hepatocellular carcinoma. While brachyury is promoting epithelial-mesenchymal transition, it can also induce metastasis of Hepatocellular carcinoma cells. Brachyury expression is a prognostic biomarker for Hepatocellular carcinoma, and the gene may be a target for cancer treatments in the future. Additionally, overexpression of brachyury may play a part in epithelial-mesenchymal transition associated with benign disease such as renal fibrosis.

Presentation

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

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.

<i>Catalog No.</i>	<i>Quantity</i>
BSB-9036-CS	5 slides
BSB 3496	5 slides

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

- Entrez Gene: T T brachyury transcription factor [Homo sapiens (human)]
<https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=6862>
- Edwards YH, et al. The human homolog T of the mouse T (Brachyury) gene; gene structure, cDNA sequence, and assignment to chromosome 6q27". Genome Res. 1996; 6 (3): 226-33.
- Scholz CB and Technau U. The ancestral role of Brachyury: expression of NemBra1 in the basal cnidarian Nematostella vectensis (Anthozoa). Dev. Genes Evol. 2003; 212 (12): 563-70.
- Le Gouar, M, et al. Expression of a SoxB and a Wnt2/13 gene during the development of the mollusc Patella vulgata. Development genes and evolution. 2004; 214(5): 250-256.
- Lartillot, N; et al. Expression pattern of Brachyury in the mollusc Patella vulgata suggests a conserved role in the establishment of the AP axis in Bilateria.". Development. 2002; 129 (6): 1411-1421.
- Kromik A, et al. The mammalian cervical vertebrae blueprint depends on the T (brachyury) Gene. Genetics. 2015; 199 (3):183+.
- Vujovic S, et al. Brachyury, a crucial regulator of notochordal development, is a novel biomarker for chordomas. J. Pathol. 2006; 209 (2): 157-65.
- Sun SR, et al. The T-box transcription factor Brachyury promotes renal interstitial fibrosis by repressing E-cadherin expression. J Cell Commun Signal. 2014; 12:76.
- 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