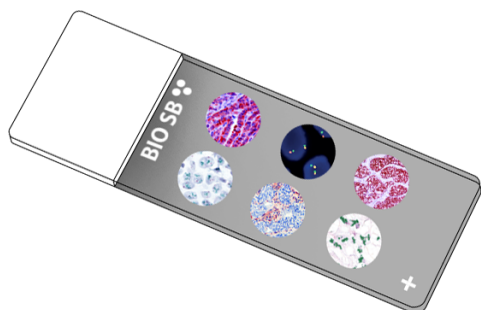


## NKX2.2 Control Slides



### Intended Use

For In Vitro Diagnostic Use.

### Summary and Explanation

Homeobox protein NKX2.2 is a protein encoded by the NKX2-2 gene. NKX2.2 is a homeodomain-containing transcription factor that plays a critical role in neuroendocrine/glial differentiation. NKX2.2 is expressed in the developing forebrain and spinal cord. Functionally, the transcription factor is thought to be involved with neuronal developing, patterning, and fate specification of neurons and oligodendrocytes.

NKX2.2 was recently reported as a valuable marker for Ewing's sarcoma. The vast majority of Ewing's sarcomas (85%) harbor a chromosomal translocation, most commonly t(11;22)(q24;q12) encoding an aberrant EWS-FLI transcription factor. NKX2.2 expression is tightly correlated with EWS-FLI expression, a critical downstream target that is required for the cancerous behavior of Ewing's sarcoma. While CD99 is the classical marker for Ewing's sarcoma diagnosis, it is relatively nonspecific. In addition to Ewing's sarcoma, CD99 is also expressed on lymphocytes, hematopoietic cells, endothelial cells and a variety of tumors. In contrast, NKX2.2 labels 93% of Ewing's sarcoma and only a small subset (14/130) of non-Ewing tumors, demonstrating a sensitivity of 93% and specificity of 89%. Staining with NKX2.2 can aid in the differential diagnosis of small round cell tumors.

### Presentation

Five slides of NKX2.2 positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

| <i>Catalog No.</i> | <i>Quantity</i> |
|--------------------|-----------------|
| BSB-9308-CS        | 5 slides        |
| BSB 3112           | 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<br>AP/HRP | PolyDetector<br>AP/HRP | PolyDetector<br>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. Price M, et al. Regional expression of the homeobox gene Nkx-2.2 in the developing mammalian forebrain". Neuron. 1992; 8 (2): 241-55.
2. Tochitani S, Hayashizaki Y. Nkx2.2 antisense RNA overexpression enhanced oligodendrocytic differentiation. Biochemical and Biophysical Research Communications. 2008; 372 (4): 691-6.
3. Lovrics A, et al. Boolean modelling reveals new regulatory connections between transcription factors orchestrating the development of the ventral spinal cord. PLOS ONE. 2014; 9(11): e111430.
4. Yoshida A, et al. NKX2.2 is a useful immunohistochemical marker for Ewing sarcoma. Am J Surg Pathol. 2012; 36(7):993-9.
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

|                      |                                                                                                          |                                                                                                                                                                                             |                                                                                                                                           |            |                                                           |
|----------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------------------------------------------------|
| <b>EC</b> <b>REP</b> | QAdvis EAR AB<br>Ideon Science Park<br>Scheelevägen 17<br>SE-223 70 Lund, Sweden                         |  Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich                           |  Manufacturer<br>Fabricant<br>Hersteller               | <b>REF</b> | Catalog Number<br>Référence du catalogue<br>Bestellnummer |
| <b>IVD</b>           | In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro<br>In-Vitro-Diagnostikum |  Read Instructions for Use<br>Consulter les instructions<br>d'utilisation<br>Gebrauchsanweisung beachten |  Expiration Date<br>Utiliser jusqu'à<br>Verwendbar bis | <b>LOT</b> | Lot Number<br>Code du lot<br>Chargenbezeichnung           |