Antibody Type: Tinto Prediluted
Clone: BSB-108
Isotype: IgG2a/K
Localization: Nuclear
Species Reactivity: Human, Mouse, Rat

**Intended Use**
For Research Use Only.

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.

**Immunogen**
Synthetic peptide corresponding to the C-terminus of the human ATRX protein.

**Summary and Explanation**
ATRX is also known as ATP-dependent helicase ATRX, X-linked helicase II, or X-linked nuclear protein (XNP) is a protein that in humans is encoded by the ATRX gene. Mutations of the ATRX gene are associated with an X-linked mental retardation (XLMR) syndrome most often accompanied by alpha-thalassemia (ATRX) syndrome.

Mutation/loss of ATRX expression has been described in anaplastic gliomas. A study explored the role of ATRX status in the molecular classification of anaplastic gliomas and its impact on survival. Loss of ATRX expression was detected in 45% of anaplastic astrocytomas (AA), 27% of anaplastic oligoastrocytomas (AOA) and 10% of anaplastic oligodendrogliomas (AO). Survival analysis showed a marked separation of IDH mutant astrocytic tumors into two groups based on ATRX status: tumors with ATRX loss had a significantly better prognosis.

Another study conducted an immunohistochemical analysis of ATRX expression in adult diffuse gliomas and found ATRX immunoreactivity of tumor cells was either almost totally absent or completely retained in all cases. There was perfect concordance between the IHC results and ATRX mutation status. ATRX loss was observed in 34.3, 30.8 and 0.0% of grades II/III astrocytomas, oligoastrocytomas and oligodendrogliomas, respectively, and 12.7% of glioblastomas. Another recent study analyzed the use of ATRX, IDH and 1p/19q codeletion in a series astrocytomas, oligodendrogliomas, oligoastrocytomas and glioblastomas and presented an algorithm based on stepwise analysis with initial immunohistochemistry for ATRX and IDH1-R132H followed by 1p/19q analysis then by IDH sequencing, which reduces the number of molecular analyses and which has a far better association with patient outcome.

ATRX 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.

**Presentation**
ATRX 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.

**Preservations**
- **Catalog Num.**
  - BSB 3293: Tinto Prediluted
  - BSB 3294: Tinto Prediluted
  - BSB 3295: Tinto Prediluted
  - BSB 3296: Concentrated
  - BSB 3297: Concentrated
  - BSB 3298: Concentrated
  - BSB 3299: Control Slides

- **Dilution**
  - Ready-to-Use
  - 1:50 - 1:200

- **Volume/Qty**
  - 3.0 mL
  - 7.0 mL
  - 15.0 mL
  - 0.1 mL
  - 0.5 mL
  - 1.0 mL
  - 5 slides

**Precautions**
1. For professional users only. Ensure results are interpreted by a medical professional.
2. 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" (7).

**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 ImmunoDNA 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. De-paraffinize, 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**

<table>
<thead>
<tr>
<th>Step</th>
<th>ImmunoDetector AP/HRP</th>
<th>PolyDetector AP/HRP</th>
<th>PolyDetector Plus HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase/AP Blocker</td>
<td>5 min.</td>
<td>5 min.</td>
<td>5 min.</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
</tr>
<tr>
<td>1st Step Detection</td>
<td>10 min.</td>
<td>30-45 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>2nd Step Detection</td>
<td>10 min.</td>
<td>Not Applicable</td>
<td>15 min.</td>
</tr>
<tr>
<td>Substrate-Chromogen</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
</tr>
<tr>
<td>Counterstain</td>
<td>Varies</td>
<td>Varies</td>
<td>Varies</td>
</tr>
</tbody>
</table>

**Symbol Key / Légende des symboles/Erläuterung der Symbole**

- Storage Temperature / Température de stockage / Zulassiger temperaturbereich
- Manufacturer / Fabricant / Hersteller
- Read Instructions for Use / Consulter les instructions d'utilisation / Gebrauchsanweisung beachten
- Expiration Date / Utiliser jusque / Verwendbar bis
- Catalog Number / Référence du catalogue / Bestellnummer
- Lot Number / Code du lot / Chargenbezeichnung

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**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**