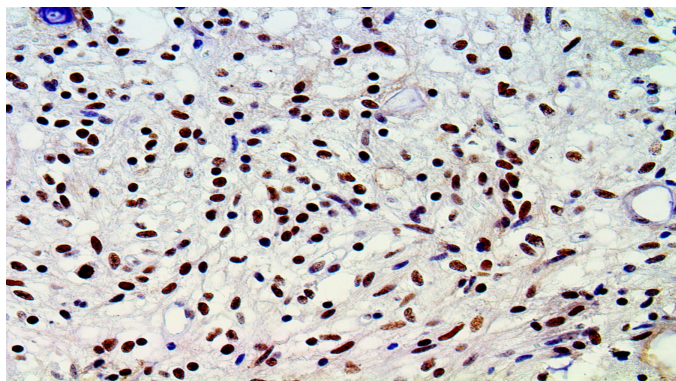


ATRX

Clone: BSB-108
Mouse Monoclonal



Inset: IHC of ATRX on a FFPE Astrocytoma 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.

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 54.5, 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.

Antibody Type	Mouse Monoclonal	Clone	BSB-108
Isotype	IgG2a/K	Reactivity	Paraffin, Frozen
Localization	Nuclear	Species Reactivity	Human, Mouse, Rat
Control	Breast, Tonsil, Testis, Salivary Gland, Placenta, Transitional Cell Carcinoma, Astrocytoma		
Application	Neural & Neuroendocrine Cancer		

Presentation

Anti-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.

<i>Catalog No.</i>	<i>Presentation</i>	<i>Dilution</i>	<i>Volume</i>
BSB 3293	Predilute	Ready-to-Use	3.0 mL
BSB 3294	Predilute	Ready-to-Use	7.0 mL
BSB 3295	Predilute	Ready-to-Use	15.0 mL
BSB 3296	Concentrate	1:50-1:200	0.1 mL
BSB 3297	Concentrate	1:50-1:200	0.5 mL
BSB 3298	Concentrate	1:50-1:200	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB-9022-CS	5 slides

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

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN₃) as a preservative. 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. 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).

Stability

This product is stable up to the expiration date on the product label.

Do not use after expiration date listed on the 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 on acetone-fixed frozen sections and acetone-fixed cell preparations.

IHC Protocol

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 IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
 8. Wash slides with ImmunoDNA washer or DI water.
 9. Continue IHC 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. Stayton CL, et al. Cloning and characterization of a new human Xq13 gene, encoding a putative helicase". Hum Mol Genet. 1995; 3 (11): 1957-64.
2. Gibbons RJ, et al. X-linked alpha-thalassemia/mental retardation (ATR-X) syndrome: localization to Xq12-q21.31 by X inactivation and linkage analysis. Am J Hum Genet. 1992; 51 (5): 1136-49.
3. Cardoso C, et al. Specific interaction between the XNP/ATR-X gene product and the SET domain of the human EZH2 protein". Hum. Mol. Genet. 1998; 7 (4): 679-84.
4. Wiestler B, et al. ATRX loss refines the classification of anaplastic gliomas and identifies a subgroup of IDH mutant astrocytic tumors with better prognosis. Acta Neuropathol. 2013; 126:443-451
5. Ikemura M, et al. Utility of ATRX immunohistochemistry in diagnosis of adult diffuse gliomas. Histopathology. 2016 Aug; 69 (2):260-7.
6. Reuss DE, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. Acta Neuropathol. 2015 Jan;129(1):133-46.
7. 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

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
	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 Number Code du lot Chargenbezeichnung