

ATRX

Concentrated and Prediluted Polyclonal Antibody
901-3251-051822

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Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3251 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	API 3251 AA	6.0 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3251 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3251 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

For In Vitro Diagnostic Use

ATRX is a rabbit polyclonal antibody that is intended for laboratory use in the qualitative identification of ATRX protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

ATRX plays a role in chromatin regulation and maintenance of telomeres. It regulates incorporation of histone H3.3 into telomeric chromatin (1). ATRX is also a major component of various essential cellular pathways such as DNA replication and repair, chromatin higher-order structure regulation, gene transcriptional regulation, etc. (2) ATRX loss was observed in grades II/III astrocytomas, oligoastrocytomas, oligodendrogliomas, and glioblastomas. In grades II/III gliomas, most ATRX- loss cases had IDH1/2 mutations (3). ATRX mutations accompanied by an alternative lengthening of telomeres (ALT), impacted favorable survival of patients with astrocytic tumors (4). Assessment of ATRX loss by immunohistochemical staining captures the majority of mutations, indicating that the use of immunohistochemical testing in routine neuropathology diagnostics gives a reasonable sensitivity (5). ATRX mutation is also detected in neuroblastoma, osteosarcoma, and pancreatic neuro-endocrine tumors (6).

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Rabbit polyclonal

Species Reactivity: Human; others not tested

Clone: N/A

Isotype: IgG

Protein Concentration: Lot specific Ig concentration is not available.

Epitope/Antigen: ATRX

Cellular Localization: Nuclear

Positive Tissue Control: Normal prostate

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protocol Recommendations (intelliPATH FLX and manual use)

Cont'd:

Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a tertiary polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluig Solution for 1 minute. Rinse with deionized water.

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (Ventana BenchMark ULTRA):

AVI3251 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 64 minutes

Peroxidase: Pre-Primary Peroxidase Inhibitor

Primary Antibody: 32 minutes, 36°C

Protocol Recommendations (Q Series – For Leica BOND-III):

ALI3251 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Protocol Name: IHC Protocol F

Detection: Bond Polymer Refine

HIER: 20 min with ER1

Peroxide Block: 5 min

Marker (Primary Antibody): 15 min

Post Primary: 8 min

Polymer: 8 min

Mixed DAB Refine: 10 min

Hematoxylin: 5 min

Performance Characteristics:

Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

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Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (8)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Lu HC, *et al.* Aberrant ATRX protein expression is associated with poor overall survival in NF1-MPNST. *Oncotarget*. 2018 May; 9:23018-28.
2. Haase S, *et al.* Mutant ATRX: uncovering a new therapeutic target for glioma. *Expert Opin Ther Targets*. 2018 Jul;22(7):599-613.
3. Ikemura M, *et al.* Utility of ATRX immunohistochemistry in diagnosis of adult diffuse gliomas. *Histopathology*. 2016 Aug;69(2):260-7.
4. Cai J, *et al.* Detection of ATRX and IDH1-R132H immunohistochemistry in the progression of 211 paired gliomas. *Oncotarget*. 2016 Mar;7(13):16384-95.
5. Cai J, *et al.* ATRX, IDH1-R132H and Ki-67 immunohistochemistry as a classification scheme for astrocytic tumors. *Oncoscience*. 2016 Sep ;3(7-8):258-65.
6. Koschmann C, *et al.* ATRX loss promotes tumor growth and impairs nonhomologous end joining DNA repair in glioma. *Sci Transl Med*. 2016 Mar;8(328):328ra28.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Astrocytoma	16	36
Glioblastoma	4	7
Ovary Cancer	0	2
Breast Cancer	13	27
Colon Cancer	8	40
Lung Cancer	24	52
Prostate Cancer	16	42
Adrenocortical Carcinoma	0	1
Bladder Cancer	0	2
Meningioma	0	3
Squamous Cell Carcinoma (esophagus)	0	2
Adenocarcinoma (stomach)	1	3
Adenocarcinoma (small intestine)	0	1
Adenocarcinoma (colon & rectum)	1	6
Kidney Cancer	0	2
Liver Cancer	0	4
Lymphoma	0	3
Adenocarcinoma (head & neck, oral cavity, hard palate)	0	1
Squamous Cell Carcinoma (head & neck, oral cavity, tongue)	1	1
Nasopharyngeal Carcinoma	0	1
Adenocarcinoma (pancreas)	0	1
Adenocarcinoma (prostate)	1	2
Adenoid Cystic Carcinoma	0	1
Squamous Cell Carcinoma (skin)	1	1
Head & Neck Nasal Cavity (melanoma)	0	1
Seminoma	0	2
Thyroid Cancer	0	2
Cervical Cancer	0	2
Endometrium Cancer	1	2
Osteosarcoma	0	1
Chondrosarcoma	0	1

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Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	5	11
Cerebellum	2	3
Adrenal	1	3
Ovary	3	3
Pancreas	3	4
Parathyroid	3	3
Pituitary	2	2
Testis	3	4
Thyroid	3	4
Breast	2	4
Spleen	3	3
Tonsil	3	3
Thymus	2	2
Bone Marrow	1	3
Lung	2	3
Heart	0	3
Esophagus	3	4
Stomach	3	4
Small Intestine	2	3
Colon	4	12
Liver	2	3
Salivary Gland	3	3
Kidney	3	4
Prostate	2	12
Uterus	2	2
Cervix	2	2
Skeletal Muscle	1	3
Skin	2	3
Peripheral Nerve	1	2
Lining Cells	2	3
Bladder	0	1
Head, Neck and Salivary Gland	0	1
Lymph Node	0	1