ATRX

Concentrated and Prediluted Polyclonal Antibody
902-3251-022822

BIOCARE MEDICAL

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

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

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 2ºC to 8ºC. 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 2ºC to 8ºC.

Staining Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
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.

Staining Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:
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 Bluing 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.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):
AVR3251 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

Staining Protocol Recommendations (Q Series – For Leica BOND-III):
ALR3251 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:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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 (NaN_3) 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)

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60 Berry Drive
Pacheco, CA 94553
USA

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Precautions Cont'd:
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.

Technical Support:
Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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.

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

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