

ATRX (Clone: D5) Mouse Monoclonal Antibody

PRODUCT INFORMATION:

| REF | |
|--------|--------------------|
| PM229 | 6ml Ready to use |
| PM229 | 3ml Ready to use |
| CM229 | 1ml Concentrated |
| CM229 | 0.5ml Concentrated |
| CM229 | 0.1ml Concentrated |
| HAM229 | 6ml Ready to use |
| HAM229 | 3ml Ready to use |

PERFORMANCE CHARACTERISTICS:

Localization: Nucleus
Retrieval Buffer: Tris- EDTA, pH 9.0
Incubation: 30-60 minutes
Positive control: Astrocytoma, Glioma

upright position. The contents of the vial should be used within 9 months from the opening of the vial.

SPECIMEN PREPARATION

Staining Recommendations:

Routinely processed, FFPE tissues are suitable for use with this primary antibody, when using PathnSitu's Poly Excel HRP/DAB detection system. The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification. The thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as the antigenicity of the cut sections may diminish over a period of time. It is recommended to stain known positive and negative controls simultaneously with unknown specimens.

INTENDED USE

For *in vitro* diagnostic use only

This antibody is intended for use in qualitatively identifying ATRX antigen by light microscopy in formalin fixed, paraffin embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Interpretation of any positive or negative staining must be complemented with the evaluation of proper known controls (Positive and Negative) and must be made within the context of the patient's clinical history and other diagnostic tests. A qualified and trained pathologist must perform the evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using non-immunologic histochemical stains.

PRECAUTIONS

1. This product should be used by qualified and trained professional users only
2. The product contains < 0.1% of sodium azide as a preservative and is not classified as hazardous, refer to MSDS for further details
3. As with any product derived from biological sources, proper handling procedures should be used
4. Do not use reagents after the expiration date
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed of according to local state and federal regulations
7. Avoid microbial contamination of reagents as it may lead to incorrect results

SUMMARY AND EXPLANATION

ATRX is a member of the Snf2 family of helicase/ATPases, which contribute to the remodeling of the nucleosome structure in an ATP-dependent manner, and facilitate the initiation of transcription and replication. Structurally, ATRX contains a PHD zinc finger motif. ATRX is regulated throughout the cell cycle where it is differentially distributed within the nucleus. During interphase, ATRX predominately associates with the nuclear matrix, while during mitosis, ATRX localizes with condensed chromatin. At the onset of M phase, phosphorylation rapidly induces this redistribution of ATRX to the short arms of human acrocentric chromosomes, where it then specifically complexes with hetero-chromatin protein 1 α to mediate chromosomal segregation. Mutations in the ATRX gene correlate with a high incidence of severe X-linked form of syndromal mental retardation associated with a thalassaemia or ATRX syndrome.

STAINING PROCEDURE

Antigen Retrieval Solution: Use Tris-EDTA Buffer (Cat#PS009) as an antigen retrieval solution.

Heat Retrieval Method: Retrieve sections under steam pressure for 15 minutes using PathnSitu's MERS (Multi Epitope Retrieval System) for optimal retrieval of the epitopes, allow solution to cool at room temperature, transfer the tissue sections/slides to the distilled water prior to the primary antibody application.

Primary Antibody: Cover the tissue sections with primary antibody and incubate for 30-60 min at room temperature when used PathnSitu's PolyExcel Detection System.

Detection System: Refer to PathnSitu's PolyExcel HRP/ DAB detection system protocol for optimal staining results.

PRINCIPLE OF THE PROCEDURE

The identification of the antigen on the FFPE tissues is carried out using the above stated antibody. The antigen and antibody complex are visualized using an enzyme coupled (HRP/AP) secondary antibody with specific binding to the primary antibody, this complex is visualized by the enzymatic activation of the chromogen resulting in a visible reaction production of the antigenic site. Each and every step involves precise time and optimal temperature and the results are interpreted using a light microscope by a qualified and trained pathologist.

QUALITY CONTROL

The recommended positive tissue control for ATRX are Astrocytoma and Glioma. A positive and negative tissue controls must be run with every staining procedure performed for monitoring the correct performance of processed tissue and test reagents. A negative tissue control provides an indication of non-specific background staining. If the results are not expected in positive and negative controls, the test must be considered invalid and the entire procedure must be cross verified. Individual laboratory must establish their own quality control to validate the process and antibody when opening a vial.

REAGENT PROVIDED

Concentrated format: ATRX antibody is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% sodium azide (NaN₃).

Recommended dilutions: 1:50 – 1:100

The antibody dilution and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by individual laboratories.

Pre-diluted format: PathnSitu's ready to use antibodies are pre-titrated to optimal staining conditions. Further dilution will affect the efficacy of the antibody and may yield to sub-optimal staining.

Immunogen: ATRX (D-5) is a mouse monoclonal antibody raised against amino acids 2193-2492 mapping near the C-terminus of ATRX of human origin.

Host, Isotype: Mouse, IgG2a, K.

INTERPRETATION OF RESULTS

ATRX stains the Nucleus. A qualified experienced/trained pathologist must interpret the results in the patient's sample along with the positive and negative controls.

PERFORMANCE CHARACTERISTICS

PathnSitu products will undergo thorough a quality control check before it is released to the market. The antibody showed consistent specific and sensitive staining on the multiple positive tissue controls tested, by inter run, intra run and lot based studies. The antibody is stable for the expiry mentioned on the labels which is determined by real time or accelerated methods.

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8°C. When stored at the appropriate conditions, the antibody is stable until expiry. Do not use the antibody after the expiration date provided on the vial in any condition.

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vial into the refrigerated conditions in an

TROUBLESHOOTING

1. Follow the antibody specific protocol recommendations according to the data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, antibody freezing and thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping or inaccurate results

DS-PM229-B

Page 1 of 2

3. Do not allow the section to dry out during the entire IHC process
4. Excessive or incomplete counter staining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu's Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

Authorized and skilled/trained personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified trained pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

BIBLIOGRAPHY

1. Eid, R., et al. 2015. Genetic inactivation of ATRX leads to a decrease in the amount of telomeric cohesin and level of telomere transcription in human glioma cells. *Mol. Cell. Biol.* 35: 2818-2830.
2. Levy, M.A., et al. 2015. ATRX promotes gene expression by facilitating transcriptional elongation through guanine-rich coding regions. *Hum. Mol. Genet.* 24: 1824-1835.
3. Pacurari, M., et al. 2013. The microRNA-200 family targets multiple non- small cell lung cancer prognostic markers in H1299 cells and BEAS-2B cells. *Int. J. Oncol.* 43: 548-560.

EXPLANATION OF SYMBOLS



Lot number / Batch number



Expiry



In vitro diagnostic use



Storage limitation



Date of manufacture



Catalogue number



EU compliance