PLAP (Clone: PRM136)
Rabbit Monoclonal Antibody

RUO

PRODUCT INFORMATION:

REF

MR1493	**6ml Ready to use**
MR1493	**3ml Ready to use**
MRC1493	**1ml Concentrated**
MRC1493	**0.5ml Concentrated**
MRC1493	**0.1ml Concentrated**
MRH1493	**6ml Ready to use**
MRH1493	**3ml Ready to use**

PERFORMANCE CHARACTERISTICS:

Localization: Membrane/Cytoplasm
Retrieval Buffer: Tris-EDTA, pH 9.0
Incubation: 30-60 minutes
Positive control: Seminoma & Placenta

INTENDED USE

This antibody is intended for use in qualitatively identifying PLAP antigen by light microscopy in formalin-fixed, paraffin-embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Any observed staining or its absence should be reviewed alongside appropriate positive and negative controls. Interpretation must be carried out by qualified personnel trained in histological and molecular techniques. This product is for research use only and not intended for diagnostic or therapeutic use.

SUMMARY AND EXPLANATION

Alkaline phosphatases (ALP) are dimeric enzymes by glycosylphosphatidylinositol anchors to the cell membrane. There are at least four distinct but related isozymes: placenta ALP (PLAP), germ cell ALP (PLAP- like or GCAP), intestinal ALP (IAP) and non-specific tissue ALP (TNAP). These isozymes may serve to guide migratory cells, to transport specific molecules such as fat and immunoglobulins across membranes or to detoxify lipopolysaccharide and prevent bacterial invasion across the gut mucosal barrier. This antibody specifically recognizes PLAP and GCAP. PLAP is expressed in the human placenta beginning late in the first trimester of pregnancy. GCAP is expressed in normal endocervix and fallopian tube. Ectopic expression of GCAP is associated with germ cell tumors: intratubular germ cell neoplasia, unclassified (IGCNU), seminoma, embryonal carcinoma and choriocarcinoma. PLAP has been used as a marker for germ cell tumor. PLAP is also known as Placental Alkaline Phosphatase, LNPEP, ALPP, PLAA.

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. Every step involves precise time and optimal temperature and the results are interpreted using a light microscope by a qualified and trained personnel.

REAGENT PROVIDED

Concentrated format: PLAP is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide (NaN_3).
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-tittered to optimal staining conditions. Further dilution will affect the efficacy of the antibody and may yield to sub-optimal staining.
Immunogen: A peptide corresponding to the N-terminus of human PLAP (Placental alkaline phosphatase)
Host, Isotype: Rabbit, IgG

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 in an upright position in refrigerated conditions. 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 cut sections' antigenicity may diminish over time. Staining known positive and negative controls simultaneously with unknown specimens is recommended.

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 contamination of reagents as it may lead to incorrect results

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 before 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.

QUALITY CONTROL

The recommended positive tissue controls for PLAP are Seminoma and placenta. A positive and negative tissue controls must be run with every staining procedure performed to monitor 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. The individual laboratory must establish their own quality control to validate the process and antibody when opening a vial.

INTERPRETATION OF RESULTS

PLAP stains the Membrane or Cytoplasm. A qualified experienced/trained personnel must interpret the results in the patient's sample along with the positive and negative controls.

ANALYTIC PERFORMANCE CHARACTERISTICS

1. Heat the paraffin-embedded tissue slides for a suitable duration at an appropriate temperature to promote tissue adhesion.

Note: Use positively charged coated slides (Cat no.: PS-011-72) for better adherence.

2. Deparaffinize the slides using xylene (preferably 3 changes with 5min each) to clear the paraffin wax present on and around the tissue.
3. Rehydrate the slides in graded alcohols (100%, 70%, and 50%) for 3 min each and in distilled water (preferably 2 changes with 2 min each) respectively.
4. Immerse the slides in 1X retrieval buffer (preferable Cat No.: PS009) and subject them to Heat-induced epitope retrieval by using a multi-epitope retrieval system (MERS-i) to unmask the epitopes.
5. Proceed further by using Poly Excel DAB Detection system (preferably Cat no: PEH002 or OSH001) kit components like Poly Excel Peroxidase Block to inactivate or block the non-specific binding firstly.

US Office: 7026 Koll Center Parkway, STE#211, Pleasanton, CA-94566, USA Ph: +1 925-218-6939, 040-2701-5544
India Office:PS Heights, Plot# A-28/1/11B/B, Road#, 15, Industrial Development Area, Nacharam, Secunderabad-76
Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com

6. Apply the primary antibody specific to the target antigen. Incubate slides with the primary antibody for a suitable duration at an appropriate temperature as mentioned in the datasheet.
7. Rinse the slides to remove unbound primary antibody using wash buffer (preferably Cat no: PS006)
8. Apply the secondary antibody (preferably Poly Excel Poly HRP- Anti-Mouse/Anti-Rabbit Cat no: PEH002 or OSH001) conjugated to an enzyme that recognizes the primary antibody. Incubate slides with the secondary antibody for a suitable duration at an appropriate temperature.
9. Rinse the slides to remove unbound secondary antibodies using wash buffer (Preferably Immunowash buffer Cat no: PS006)
10. Apply a substrate, PolyExcel Stunn DAB Chromogen for enzyme-conjugated secondary antibody for a suitable duration.
11. Counter-stain the tissue section to visualize the expression in specific structures or cell types.
12. Dehydrate slides through graded alcohols (70%, 90%, 100%,100%), clear the slides in Xylene (preferably 3 changes with 2min each) and mount the slides with an appropriate mounting medium.
13. Visualize the stained slides under the microscope.

The antibody consistently exhibited specific and sensitive staining across various positive tissue controls including Seminoma and Placenta tissue samples with membrane or cytoplasm staining whereas no staining was observed in the negative control, Testis. This specificity and sensitivity were validated through inter-run, intra-run, and lot-based studies. The stability of the antibody which was determined using real-time or accelerated methods extends until the expiration date indicated on the product labels.

TROUBLESHOOTING

1. Follow the antibody-specific protocol recommendations according to the datasheet 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
3. Do not allow the section to dry out during the entire IHC process
4. Excessive or incomplete counterstaining 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

1. This product is intended for use only by trained and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. In instances where the staining pattern or localisation differs from the specifications outlined in this datasheet, please get in touch with technical support for guidance.
11. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. This product is intended for single-use applications only. Once applied to a tissue section, reagents should not be recovered or reused, as this may compromise test integrity and specificity.
14. Performance characteristics may vary slightly between lots; users should validate each new lot with controls before routine use.
15. Results may vary depending on instruments, detection systems, or protocol variations. Users must verify performance under their specific conditions.
16. Do not use after the expiry date stated on the label, as performance cannot be guaranteed
17. Improper fixation, over-fixation, or under-fixation may affect staining quality.
18. PathnSitu makes no warranties beyond those expressly stated in the product description.
19. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
20. Please refer to the complete data sheet for all instructions, precautions, and additional product limitations.
21. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS)

BIBLIOGRAPHY

1. Millan JL, et al.: Crit Rev Clin Lab Sci 1995, 32:1-39

2. Fishman L, et al.: Cancer Res 1976, 36:2268-2273

EXPLANATION OF SYMBOLS

LOT **Lot number / Batch number**

Expiry

RUO ***Research* use only**

Storage limitation

Date of manufacture

REF **Catalogue number**

Manufacturer address

US Office: 7026 Koll Center Parkway, STE#211, Pleasanton, CA-94566, USA Ph: +1 925-218-6939, 040-2701-5544
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