

**Histone H3 Phospho (pT3)-(Clone: EP233)  
Rabbit Monoclonal Antibody**

<b>PRODUCT INFORMATION:</b>	<b>PERFORMANCE CHARACTERISTICS:</b>
MR1220 6ml Ready to use	<b>Localization:</b> Nucleus
MR1220 3ml Ready to use	<b>Retrieval Buffer:</b> Tris-EDTA, pH 9.0
MRC1220 1ml Concentrated	<b>Incubation:</b> 30-60 mins
MRC1220 0.5ml Concentrated	<b>Positive control:</b> Breast Ca, Tonsil
MRC1220 0.1ml Concentrated	

**INTENDED USE**

**For research use only**  
This antibody is intended for use in qualitatively identify Histone H3 Phospho (pT3) 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 evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains

**SUMMARY AND EXPLANATION**

Histone H3 is a core histone protein, which complexes with the other histones to form the major constituents of chromatin in eukaryotic cells. In mammalian cells, phosphorylation of Threonine 3 residue in histone H3 reaches a maximum for condensation during mitosis. Phosphorylation of histone H3 (pHH3) occurs only during late G2 phase and mitosis. pHH3 is a marker for mitoses in various types of tumors. It is particularly useful in identifying mitotic figures in tumors with dense cellularity, limited mitotic activity, and/or abundant apoptotic, pyknotic or distorted nuclei.

**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 is 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 to 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.

**REAGENT PROVIDED**

**Concentrated format:** Antibody to Histone H3 Phospho (pT3) is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide (NaN3).  
**Recommended dilutions:** 1:25 – 1:50  
The antibody dilution and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by individual laboratory.  
**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:** A synthetic phospho-peptide corresponding to residues surrounding Thr3 of Human Histone H3  
**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 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 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 used 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. Thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as 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.

**PRECAUTIONS**

1. This product should be used by qualified and trained professional users only
2. The product contains < 0.1% of sodium azide as preservative and is not classified hazardous, refer 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 expiration date
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed according to local state and federal regulations
7. Avoid microbial contamination of reagents as it may lead to incorrect results

**STAINING PROCEDURE**

**Antigen Retrieval Solution:** Use **Tris-EDTA Buffer (Cat#PS009)** as 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 the 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.

**QUALITY CONTROL**

The recommended positive tissue control for Histone H3 Phospho (pT3) is Tonsil and Breast Carcinoma. A positive and negative tissue control must be run with every staining procedure performed for monitoring the correct performance of processed tissue and test reagents. A negative tissue controls provide 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 entire procedure must be cross verified. Individual laboratory must establish their own quality control to validate the process and antibody when opened a vial.

**INTERPRETATION OF RESULTS**

Histone H3 Phospho (pT3) 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 a thorough 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.

**TROUBLESHOOTING**

1. Follow the antibody specific protocol recommendations according to 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
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](mailto: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. Yang GG: MLO Med Lab Obs 2011, 43:20, 23
2. Colman H, et al.: Am J Surg Pathol 2006, 30:657-664
3. Ribalta T, et al.: Am J Surg Pathol 2004, 28:1532-1536
4. Hightower E, et al.: Pituitary 2012, 15:556-561

Histone H3 Phospho (pT3), EP233 antibody has been created by Epitomics Inc., using Epitomics' proprietary rabbit monoclonal antibody technology covered under Patent No.'s 5,675,063 and 7,402,409.

#### EXPLANATION OF SYMBOLS

LOT- Lot number / Batch number



- Expiry

**RUO** Research use only



Storage limitation