

# Phospho-Histone H3

Concentrated and Prediluted Polyclonal Antibody  
901-404-090823

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CP 404 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	PP 404 AA	6.0 mL	Ready-to-use	N/A

## Intended Use:

For *In Vitro* Diagnostic Use

Phospho-Histone H3 is a rabbit polyclonal antibody that is intended for professional laboratory use in the qualitative identification of Phospho-Histone H3 (Ser10) 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:

Phospho-Histone H3 (pHH3) is present in cells undergoing mitosis. The phosphorylation of histone H3 plays an important role in gene expression, chromatin remodeling, chromosome condensation, and cell division. Histone H3 phosphorylation may initiate at different phases of cell division in different organisms, but metaphase chromosomes are always found to be heavily phosphorylated. H3 phosphorylation at serine 10 and serine 28 is coupled with mitotic chromosome segregation and condensation. The significance of the mitotic index (MI) using the Mitosis marker anti-pHH3 antibody has been reported to be of great value in breast cancer, melanoma and meningiomas. pHH3 staining may also be a useful method in which accurate determination of proliferation potential is relevant to tumor grading.

## 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- or two-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Rabbit polyclonal

**Species Reactivity:** Human; other species not tested.

**Clone:** N/A

**Isotype:** N/A

**Protein Concentration:** Lot specific Ig concentration is not available.

**Epitope/Antigen:** PhosphoSer10

**Cellular Localization:** Nuclear

**Positive Tissue Control:** Tonsil or melanoma

**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 vial label when stored under these conditions. Do not use after expiration date. Storage under any condition other than those specified must be verified. Diluted reagents should be used promptly; store any remaining reagent at 2°C to 8°C. The stability of user diluted reagent has not been established by Biocare.

## 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 product 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 secondary conjugated 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 Bluing Solution for 1 minute. Rinse with deionized water.

**Technical Note:**

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 detection system. Use TBS for washing steps.

## 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

## 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<sub>3</sub>) 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)<sup>1</sup>
2. Handle materials of human or animal origin as potentially biohazardous and dispose of such materials with proper precautions. In the event of exposure, follow the health directives of the responsible authorities where used.<sup>2,3</sup>
3. 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.<sup>4</sup>
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
6. Do not use reagent after the expiration date printed on the vial.
7. Follow local and/or state authority requirements for method of disposal.
8. The SDS is available upon request and is located at <http://biocare.net>.

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## Troubleshooting:

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

## References:

1. 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."
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
3. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
4. 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.
5. Skaland I, et al. The prognostic value of the proliferation marker phosphohistone H3 (PPH3) in luminal, basal-like and triple negative phenotype invasive lymph node-negative breast cancer. *Cell Oncol.* 2009;31(4):261-71.
6. Nasr MR, El-Zammar O. Comparison of pHH3, Ki-67, and Survivin immunoreactivity in benign and malignant melanocytic lesions. *Am J. Dermatopathol.* 2008 Apr;30(2):117-22.
7. Skaland I, et al. Phosphohistone H3 expression has stronger prognostic value than classical prognosticators in invasive lymph node-negative breast cancer patients less than 55 years of age. *Mod Pathol.* 2007 Dec; 20(12):1307-15.
8. Kim YJ, et al. Prognostic significance of the mitotic index using the mitosis marker anti-phosphohistone H3 in meningiomas. *Am J. Clin Pathol.* 2007 July; 128(1):118-25.