

## pHH3 (RM)

Concentrated and Prediluted Rabbit Monoclonal Antibody  
901-3130-091817

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

<b>Catalog Number:</b>	<b>ACI 3130 A, C</b>	<b>API 3130 AA</b>
<b>Description:</b>	0.1, 1.0 ml, concentrated	6.0 ml, prediluted
<b>Dilution:</b>	1:100	Ready-to-use
<b>Diluent:</b>	Da Vinci Green	N/A

### Intended Use:

For In Vitro Diagnostic Use

pHH3 (RM) [BC37] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of phosphohistone 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:

Phosphohistone H3 (pHH3) is a recently described marker specific for cells undergoing mitosis. Serine 10 of Histone H3 is phosphorylated in association with mitotic chromatin condensation in late G2 and M phase of the cell cycle and thus, pHH3 can distinguish mitosis from apoptotic nuclei (1). Microscopic evaluation of mitotic figures by hematoxylin and eosin (H&E) staining is a routine procedure in the assessment of the prognostic grade of tumors (2,3). The immunohistochemical (IHC) staining of pHH3 (Ser10) has been reported to be comparable to mitotic figure staining in the H&E section (4-6). However, in some cases, H&E staining may misclassify mitotic cells as apoptotic bodies or piknotic nuclei, resulting in an underestimation of the mitotic index (MI). IHC with pHH3 may provide a more accurate assessment of all mitotic cells, as well as cells in which Histone H3 has been phosphorylated immediately prior to entering prophase (7). Prognostic significance of the mitotic index using an anti-pHH3 antibody has been reported to be of great value in breast cancer, melanoma and meningiomas (8,9).

A rabbit monoclonal (RM) antibody targeting phosphorylated Serine 10 of pHH3, clone [BC37], has been developed and characterized by Western blot, ELISA, and IHC. In tonsil and melanoma, pHH3 (RM) displays stronger staining intensity in mitotic figures compared to the polyclonal pHH3. Additionally, pHH3 (RM) does not exhibit granular staining in interphase nuclei, unlike the polyclonal pHH3 (Figure 1A, 1B). pHH3 (RM) may offer other advantages common to rabbit monoclonal antibodies, including a specific epitope and lot-to-lot consistency.

### 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, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Rabbit monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** BC37

**Isotype:** IgG

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** PhosphoSer10 of Histone H3 protein

**Cellular Localization:** Nuclear (mitotic figure)

**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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

### Protocol Recommendations:

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's 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 Biocare's 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 has been standardized with Biocare's MACH 4 detection system. Use TBS buffer 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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

### 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) (10)



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### Precautions Cont'd:

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. (11)
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>.

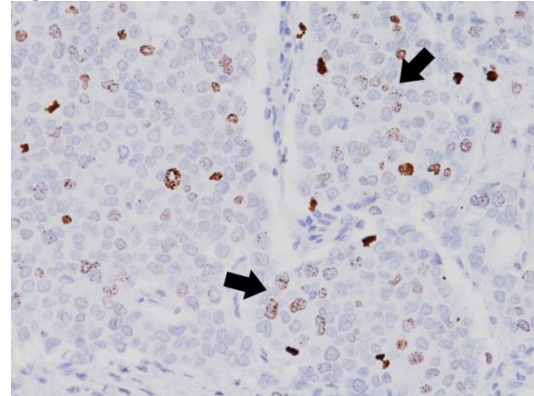
### Troubleshooting:

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

### References:

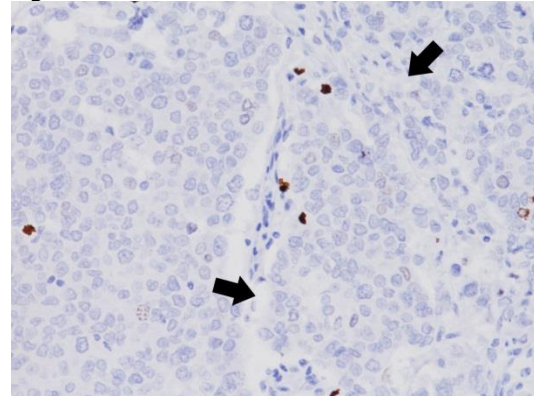
1. Ladstein RG, *et al.* Prognostic importance of the mitotic marker phosphohistone H3 in cutaneous nodular melanoma. *J Invest Dermatol.* 2012 Apr; 132(4):1247-52.
2. Jannink I, van Diest PJ, Baak JP. Comparison of the prognostic value of four methods to assess mitotic activity in 186 invasive breast cancer patients: classical and random mitotic activity assessments with correction for volume percentage of epithelium. *Hum Pathol.* 1995 Oct; 26(10):1086-92.
3. Yadav KS, *et al.* Assessment of interobserver variability in mitotic figure counting in different histological grades of oral squamous cell carcinoma. *J Contemp Dent Pract.* 2012 May 1; 13(3):339-44.
4. Thareja S, *et al.* Analysis of tumor mitotic rate in thin metastatic melanomas compared with thin melanomas without metastasis using both the hematoxylin and eosin and anti-phosphohistone 3 IHC stain. *Am J Dermatopathol.* 2014 Jan; 36(1):64-7.
5. Ikenberg K, *et al.* Immunohistochemical dual staining as an adjunct in assessment of mitotic activity in melanoma. *J Cutan Pathol.* 2012 Mar; 39(3):324-30.
6. Casper DJ, *et al.* Use of anti-phosphohistone H3 immunohistochemistry to determine mitotic rate in thin melanoma. *Am J Dermatopathol.* 2010 Oct; 32(7):650-4.
7. Veras E, *et al.* Mitosis-specific marker phospho-histone H3 in the assessment of mitotic index in uterine smooth muscle tumors: a pilot study. *Int J Gynecol Pathol.* 2009 Jul; 28(4):316-21.
8. 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.
9. 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.
10. 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."
11. 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.

Figure 1A



pHH3 rabbit polyclonal staining in melanoma. Note: Cells labeled at interphase (arrows) can be difficult to interpret and may cause inaccurate counting.

Figure 1B



pHH3 rabbit monoclonal [BC37] staining in melanoma. Note: Cells are not labeled at interphase; thus, interpretation may be easier and counting may be more accurate.