pHH3 (RM)

Concentrated and Prediluted Monoclonal Antibody
902-3130-080917

BIOCARE MEDICAL

Catalog Number:	**ACR 3130 A, C**	**APR 3130 AA**
Description:	0.1, 1.0 ml, concentrated	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use
Diluent:	Da Vinci Green	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

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 2ºC to 8ºC. 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 2ºC to 8ºC.

Staining Protocol Recommendations:
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker product 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:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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 (NaN3) 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)
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.

Technical Support:
Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

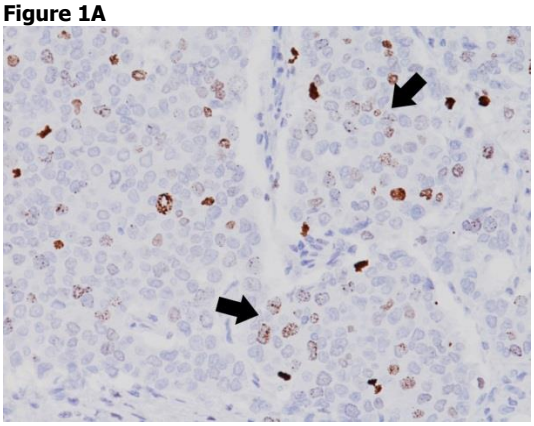
References:
1. Ladstein RG, *et al*. Prognostic importance of the mitotic marker phosphohistone H3 in cutaneous nodular melanoma. J Invest Dermatol. 2012 Apr; 132(4):1247-52.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

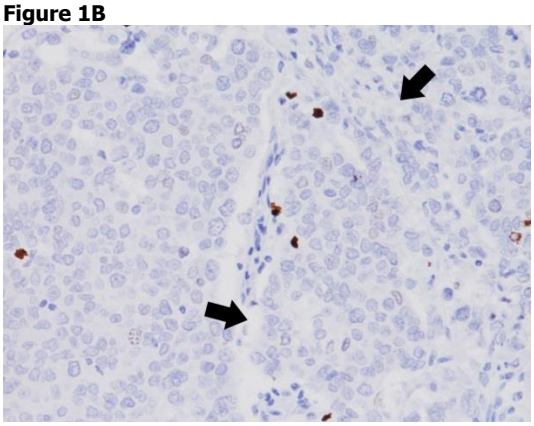
References Cont'd:
2. Jannink I, van Diest PJ, Baak JP. Comparison of the prognostic value of four methods to assess mitotic activity in 186 invasive breast cancer patients: classical and random mitotic activity assessments with correction for volume percentage of epithelium. Hum Pathol. 1995 Oct; 26(10):1086-92.
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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080