GENE BIO SOLUTION

pHH3 (RM)

Format	Catalog No.	Pack size	Dilution
Concentrated	GB3130A,C	0.1, 0.1 mL	1:100
Prediluted	GB3130AA	6.0 mL	Ready to use

PRODUCT DESCRIPTION -

Phosphohistone H3 (pHH3) is a newly identified marker exclusive to mitotic cells. Phosphorylation of Serine 10 on Histone H3 occurs during mitotic chromatin condensation in the late G2 and M phases of the cell cycle, enabling pHH3 to differentiate between mitotic and apoptotic nuclei. The microscopic examination of mitotic patterns using hematoxylin and eosin (H&E) staining is a standard practice in evaluating the prognostic grade of malignancies. The immunohistochemistry (IHC) staining of pHH3 (Ser10) has been found to be analogous to mitotic figure staining in the H&E section. Nonetheless, in certain instances, H&E staining may erroneously categorize mitotic cells as apoptotic bodies or pyknotic nuclei, leading to an underestimation of the mitotic index (MI). Immunohistochemistry with pHH3 may yield a more precise evaluation of all mitotic cells, including those in which Histone H3 has been phosphorylated just before prophase onset. The prognostic importance of the mitotic index utilizing an anti-pHH3 antibody has been demonstrated to be highly valuable in breast cancer, melanoma, and meningiomas.

A rabbit monoclonal antibody (RM) specific for phosphorylated Serine 10 of pHH3, designated clone [BC37], has been produced and described using Western blot, ELISA, and immunohistochemistry (IHC). In tonsil and melanoma, pHH3 (RM) exhibits greater staining intensity in mitotic figures than the polyclonal pHH3. Furthermore, pHH3 (RM) does not display granular staining in interphase nuclei, in contrast to the polyclonal pHH3. pHH3 (RM) may provide additional benefits typical of rabbit monoclonal antibodies, such as a particular epitope and constancy across different lots.

INTENDED USE -

pHH3 (RM) [BC37] is a rabbit monoclonal antibody designed for laboratory application in the qualitative detection of pHH3 protein using immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical assessment of any staining or its absence must be supplemented by morphological analyses with appropriate controls and should be considered in conjunction with the patient's clinical history and other diagnostic evaluations by a certified pathologist.

SUMMARY AND EXPLANATION -

Phosphohistone H3 (pHH3) is a newly identified marker exclusive to mitotic cells. Phosphorylation of Serine 10 on Histone H3 occurs during mitotic chromatin condensation in the late G2 and M phases of the cell cycle, enabling pHH3 to differentiate between mitotic and apoptotic nuclei. The microscopic examination of mitotic patterns using hematoxylin and eosin (H&E) staining is a standard practice in evaluating the prognostic grade of malignancies. The immunohistochemistry (IHC) staining of pHH3 (Ser10) has been found to be analogous to mitotic figure staining in the H&E section. Nonetheless, in certain instances, H&E staining may erroneously categorize mitotic cells as apoptotic bodies or pyknotic nuclei, leading to an underestimation of the mitotic index (MI). Immunohistochemistry with pHH3 may yield a more precise evaluation of all mitotic cells, including those in which Histone H3 has been phosphorylated just before prophase onset. The prognostic importance of the mitotic index utilizing an anti-pHH3 antibody has been demonstrated to be highly valuable in breast cancer, melanoma, and meningiomas.

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PRINCIPLE OF PROCEDURE -

Antigen detection in tissues and cells is a multi-phase immunohistochemical procedure. The first step attaches the primary antibody to its designated epitope. Following the tagging of the antigen with a primary antibody, a one- or two-step detection protocol may be utilized. The one-step approach will utilize an enzyme-conjugated polymer that attaches to the main antibody. A two-step approach will involve the addition of a secondary antibody to bind to the primary antibody. An enzyme-conjugated polymer is subsequently introduced to engage with the secondary antibody. The presence of bound antibodies is demonstrated by a colorimetric response.

SOURCE - Rabbit monoclonal

SPECIES REACTIVITY - Human; others not tested

CLONE - BC37

ISOTYPE - IgG

PROTEIN CONCENTRATION - Call for lot specific Ig concentration.

EPITOPE/ANTIGEN - PhosphoSer10 of Histone H3 protein

CELLULAR LOCALISATION - Nuclear (mitotic figure)

POSITIVE TISSUE CONTROL - Tonsil or melanoma

KNOWN APPLICATIONS - Immunohistochemistry 30-40 min. At RT. Staining of formalin-fixed tissues requires heating tissue sections in between pH 7.4 - 9.0 for 45 min at 95ºC followed by cooling at room temperature for 20 minutes.

SUPPLIED AS - Buffered saline solution, pH 6.1-7.4, containing a protein carrier and less than 0.1% sodium azide preservative.

STORAGE AND STABILITY -
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Materials required but not provided -

1) Positivetissuecontrol-Tonsilormelanoma
2) Negativecontroltissue(internalorexternal)
3) Microscopeslidesandcoverslips
4) Stainingjarsorbaths
5) Timer
6) Xyleneorxylenesubstitute
7) Ethanolorreagentalcohol
8) Deionizedordistilledwater
9) Heatingequipmentorenzymefortissuepretreatmentstep
10) Detection system
11) Chromogen
12) Wash buffer
13) Hematoxylin
14) Antibody diluents
15) Peroxide block
16) Light microscope
17) Mounting medium

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 Genebio products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

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