Diagnostic BioSystems

IHC Made Affordable

Phosphohistone H3 (PHH3)

Rabbit Polyclonal Antibody

RP168

PDR168

**This datasheet is applicable to all sizes (volume) of product. Actual product volume is indicated on vial.

Document #: DS-0613-A
Effective Date: 06/27/2018

Immunogen	Species	Primary Antibody Diluent
Phosphohistone protein n isolated from human tissue.	Rabbit	K004

Lot specific Ig concentration available upon request.

Catalog #	Description
RP168	Concentrated antibody for use with Diagnostic BioSystems PolyVue™ Plus - Two Step Detection System
PDR168	Ready to use antibody for use with Diagnostic BioSystems PolyVue™ Plus - Two Step Detection System

Intended Use

For In Vitro Diagnostic Use. This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy. Clinical interpretation of staining results should be accompanied by histological studies with proper controls. Patients' clinical histories and other relevant diagnostic tests should be utilized by a qualified person(s) when evaluating and interpreting results.

Summary and Explanation

Phosphohistone H3 (PHH3) is a core histone protein, which together with other histones, forms the major protein constituents of the chromatin in eukaryotic cells. In mammalian cells, phosphohistone H3 is negligible during interphase but reaches a maximum for chromatin condensation during mitosis.Immunohistochemical studies showed anti-PHH3 specifically detected the core protein histone H3 only when phosphorylated at serine 10 or serine 28. Studies have also revealed no phosphorylation on the histone H3 during apoptosis.PHH3 can serve as a mitotic marker to separate mitotic figures from apoptotic bodies and karyorrhectic debris, which may be a very useful tool in diagnosis of tumor grades, especially in CNS, skin, gyn., soft tissue, and GIST.

Format

Purified histone protein of rabbit antiserum containing sodium azide as a preservative.

Principles of the Procedures

Antigen detection by immunohistochemistry (IHC) is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The primary antibody may be used in IHC using manual techniques or using Diagnostic BioSystems Automated Montage 360™ Staining System.

Dilution of Primary Antibody

Diagnostic BioSystems ready to use antibodies have been optimized for use with the recommended Diagnostic BioSystems Detection System and do not require further dilution. Further dilution may result in loss of sensitivity. The user must validate any such change.

Diagnostic BioSystems concentrated antibodies must be diluted in accordance with the staining procedure when used with the recommended Diagnostic BioSystems detection system. Use of any detection methods other than the recommended systems and protocols require validation by the user. Antibody dilutions should be appropriately adjusted and verified according to the detection system used.

Materials Required But Not Provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com

Storage and Handling

Store at 2-8°C. This antibody is suitable for use until the expiration date when stored at 2-8°C. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly. Unused portions of antibody preparation should be discarded after one day.

The presence of precipitate or an unusual odor indicates that the antibody is deteriorating and should not be used.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

Specimen Collection and Preparation

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

The user is advised to validate the use of the products with their tissue specimens prepared and handled in accordance with their laboratory practices.

Precautions

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). 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 in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those

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specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

Treatment of Tissues Prior to Staining

Place the slides in the recommended antigen retrieval solution using Diagnostic BioSystems Montage Opus™ Antigen Retrieval System. Allow slides to cool down for 20 minutes prior to staining.

Staining Procedure

Refer to the following table for conditions specifically recommended for this antibody. Refer to the Diagnostic BioSystems PolyVue™ Plus—Two Step Detection System or Montage PolyVue Plus™ Auto Detection System for guidance on specific staining protocols or other requirements.

Parameter	Diagnostic BioSystems Recommendations
Positive Control	Melanoma
Concentrated Dilution	1:50-1:200
Pretreatment	EDTA (pH 8.0)
Incubation Time & Temperature	30 min @ RT
Detection System	PolyVue™ Plus - Two Step Detection System or Montage PolyVue Plus™ Auto Detection System for Montage 360 System
Tissue Type	FFPE

Quality Control

Refer to CLSI Quality Standards for Design and Implementation of Immuno-histochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011.

Troubleshooting

Contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2, techsupport@dbiosys.com or your local distributor to report unusual staining.

Cellular Localization

Nuclear (mitotic figure)

Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983). Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. Tissues containing Hepatitis B surface Antigen (HBsAg) may give a false positive with horseradish peroxidase systems (Omata et al, 1980). Improper counterstaining and mounting may compromise the interpretation of results.

Performance Characteristics

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, and 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 Diagnostic BioSystems products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

References

I. Gurley LR, *et al.* Histone phosphorylation and chromatin structure during mitosis in Chinese hamster cells. A Eur J Biochem 1978; 84:1-15
II. Hendzel MJ, *et al.* Chromatin condensation is not associated ith apoptosis. J Biol Chem 1998; 273:24470-8
III. Colman H, *et al.* Assessment and prognostic significance of mitotic index using the mitosismarker phospho-histone H3 in low and intermediate-gradde infiltrating astrocytomas. Am J Surg PathoL 2006; 30:657-64

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