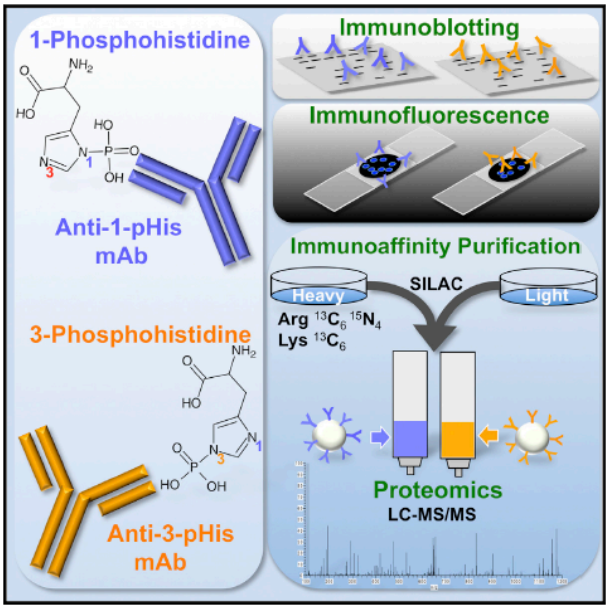
Phosphohistidine Antibodies

Isomer-specific, sequence-independent antibodies that bind phosphohistidine with high affinity.

INVENTION: Researchers in the Tony Hunter laboratory at the Salk Institute have identified monoclonal antibodies that specifically recognize peptides or proteins containing phosphorylated histidine (pHis) independently of amino-acid sequence. These antibodies can specifically recognize either the 1-pHis or 3-pHis isomer. Histidine kinases have been implicated in both solid as well as hematologic malignancies. Histidine kinases Nm23-H1 and Nm23-H2 are known to be overexpressed in various blood cancers and their overexpression correlates with poor prognosis, resistance to chemotherapy, and reduction of survival. Expression of Nm23-H1 is considered to be a prognostic indicator in malignant lymphoma, acute myelogenous leukemia, and other malignant neoplasms. This suggest that pHis antibodies may have diagnostic and therapeutic use for various types of cancer. In addition, histidine kinases are part of the two-component signal transduction system found in bacteria and in some lower eukaryotes, including some pathogenic fungi. This signaling system is critical for regulating a variety of functions, including growth, survival and virulence, and is an attractive target for the development novel antimicrobial drugs.

Summary of the related manuscript:



Histidine phosphorylation (pHis) is well studied in bacteria; however, its role in mammalian signaling remains largely unexplored due to the lack of pHisspecific antibodies and the lability of the phosphoramidate (P-N) bond. Both imidazole nitrogens can be phosphorylated, forming 1-phosphohistidine (1-pHis) or 3-phosphohistidine (3-pHis). We have developed monoclonal antibodies (mAbs) that specifically recognize 1-pHis or 3-pHis; they do not crossreact with phosphotyrosine or the other pHis isomer. Assays based on the isomer-specific autophosphorylation of NME1 and phosphoglycerate mutase were used with immunoblotting and sequencing IgG variable domains to screen, select, and characterize anti-1-pHis and anti-3-pHis mAbs. Their sequence independence was determined by blotting synthetic peptide arrays, and they have been tested for immunofluorescence staining and immunoaffinity purification, leading to putative identification of pHis-containing proteins. These reagents should be broadly useful for identification of pHis substrates and functional study of pHis using a variety of immunological, proteomic, and biological assays.

APPLICATIONS:

- Therapeutic, diagnostic, or prognostic applications for bacterial infections and specific cancers
- Drug discovery (e.g., inhibitors of histidine phosphatases or kinases)
- pHis detection

ADVANTAGES

- Specific for phosphorylated histidine
- No cross-reactivity with unphosphorylated histidine or phosphotyrosine (pTyr)
- Sequence-independent
- 1-pHis or 3-pHis isomer specific

BACKGROUND: Protein phosphorylation regulates virtually all cellular processes. While 9 out of 20 amino acids can be phosphorylated, Ser, Thr, and Tyr phosphorylation has attracted the majority of attention in eukaryotic organisms. Histidine phosphorylation has long been implicated in signal transduction (e.g., bacterial "two-component" system); however, its role remains largely unexplored in higher eukaryotes due to the difficulty in studying His phosphorylation by standard biochemical techniques. Unlike pTyr, pSer, and pThr, pHis is heat and acid liable while stable at alkaline pH. Despite the paucity of knowledge about His phosphorylation, there is growing evidence implicating His phosphorylation in various cellular processes and cancer.

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PATENT STATUS: U.S. Appl. 14/485,578 and PCT/US2015/038892
PUBLICATION: Cell. 2015 Jul 2;162(1):198-210
https://www.sciencedirect.com/science/article/pii/S0092867415006364?via%3Dihub
REFERENCE #: S12005 and S14007

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