

RayBiotech, Inc.

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Certificate of Analysis and Data Sheet

MOUSE ANTI PHOSPHOTYROSINE

With HRP-conjugated secondary antibody

Catalog No.	Species:	Isotype:
DS-MB-02120	Chemical	Mouse Ig G2b

Description

Some of the tyrosine residues can be tagged with a phosphate group (phosphorylated) by protein kinases. (In its phosphorylated state, it is referred to as phosphotyrosine). Tyrosine phosphorylation is considered as one of the key steps in signal transduction and regulation of enzymatic activity. The advent of anti-phospho-tyrosine antibodies is one of significant events in signal transduction research. Before the availability of anti-phosphotyrosine antibodies, tyrosyl phospyhorylation of proteins and enzymes was investigated through hazardous and time-consuming radioactive experiments. Anti-phosphotyrosine antibodies are commonly used in western blots after the targeted proteins have been immunoprecipitated to measure the tyrosyl phosphorylation of the proteins. Anti-phosphotyrosine antibodies are also directly used on cell lysate to examine the overall change of tyrosine phosphorylation level in response to various treatments.

Applications

Table Summary of antibody applications and working conditions

Options Functions	YES	NO	Not determined	Recommended Work dilution or concentration
ELISA	•			100ng/ml
Western Blotting	•			2ug/ml
Immunohistology - frozen			•	
Immunohistology - paraffin			•	
Immunohistology - resin			•	
Immunoprecipitation			•	
Flow Cytometry			•	
Immunofluorence staining	•			
Neutralization			•	

Note: Other applications are not tested yet. Optimal dilutions should be determined by each laboratory for each application.



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Preparation

Immunogen was recombinant protein derived from Phosphotyrosine. This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with the immunogen. The IgG fraction of tissue culture supernatant was purified by Protein affinity chromatography.

Specificity

DS-MB-02120 recognizes phosphotyrosine, enabling the detection, characterisation and isolation of proteins containing phosphorylated tyrosine residues. The phosphorylation of tyrosine acts as a important signal in the control of cell mitogenesis, differentiation, proliferation, and migration and occurs following the activation of intracellular tyrosine kinases, including the Tcell receptor (TCR), epidermal growth factor (EGF) and many families of receptor and non-receptor protein tyrosine kinases (PTKs), which catalyse the transfer of ATP to a tyrosine residue on specific cell protein targets.

Reconstitution

Supplied as 500 µg of purified antibody. It is reconstituted with 1000 µl of sterile 100mM Borate buffered saline pH8.0. Its final concentration is 0.5 mg/ml. **Please avoid freeze-thaw cycles.**

Secondary Antibody Applications

Options Functions	YES	NO	Not determined	Recommended Work dilution or concentration
Immunoassay (ELISA, Western blot)	•			1:5000-1:10000

Storage

Store at -20° C, if not intended for use within a month. Keep it at 4° C and minimize freezing and thawing when use.

References

Ruff-Jamison, S. *et al.* (1991) Heavy and light chain variable region sequences and antibody properties of anti-phosphotyrosine antibodies reveal both common and distinct features. J. Biol. Chem. 266: 6607-6613.

Takagi, S. *et al.* (1991) Intracellular localization of tyrosine kinase substrates beneath crosslinked surface immunoglobulins in B cells. J. Exp. Med. 174: 381-388.