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Phosphotyrosine Monoclonal Antibody (IG2)

Catalog Number MA4-003

Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG1
Class	Monoclonal
Type	Antibody
Clone	IG2
Immunogen	O-phospho-L-tyrosine and O-phospho-DL-tyramine
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS with 40% glycerol
Contains	0.05% sodium azide
Storage Conditions	4° C

Species Reactivity	
Species reactivity	Chemical
Published species	Chemical

Tested Applications	Dilution *
ELISA (ELISA)	1 µg/mL
Flow Cytometry (Flow)	Assay-dependent
Immunoprecipitation (IP)	2 µg
Western Blot (WB)	1:100-1:1,000
Immunocytochemistry (ICC/IF)	1:50 - 1:200

Published Applications	
Western Blot (WB)	See 1 publications below
Immunoprecipitation (IP)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

MA4-003 detects phosphotyrosine from most species and tissues. MA4-003 has been successfully used in ELISA, Western blot, Immunocytochemistry, Immunofluorescence, Immunoprecipitation, and FACS procedures. This antibody detects proteins which contain phosphorylated tyrosine residues. The MA4-003 antigen is O-phospho-L-tyrosine and O-phospho-DL-tyramine.

Background/Target Information

The role of tyrosine phosphorylation in transduction of the mitogenic signal from transmembrane receptors and in transformation by oncogene tyrosine kinases has been the subject of intense investigation for several years. While the phosphorylation of specific tyrosine residues has been shown to be a primary mechanism of signal transduction during normal mitogenesis, cell cycle progression and oncogenic transformation, its role in other areas such as differentiation and gap junction communication, is a matter of active and ongoing research. Antibodies that specifically recognize phosphorylated tyrosine residues have proved to be invaluable to the study of tyrosine -phosphorylated proteins and the biochemical pathways in which they function. The fluorescein (FITC) conjugate of clone PY20 anti-phosphotyrosine is especially useful for the detection of these P-Tyr proteins in immunohistochemical and immunocytochemical protocols in situations wherein the use of a secondary antibody would complicate detection of the protein(s) of interest.

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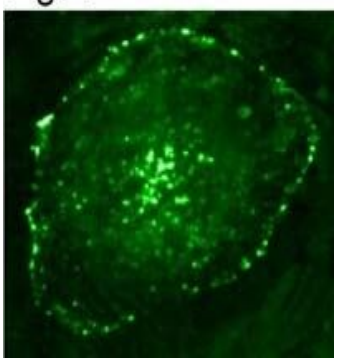
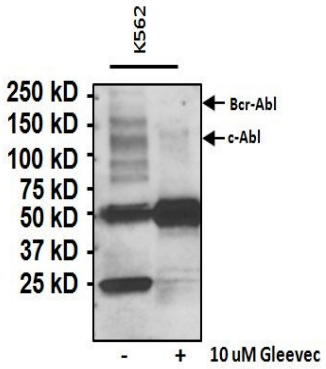
Product Images For Phosphotyrosine Monoclonal Antibody (IG2)

Fig. 1



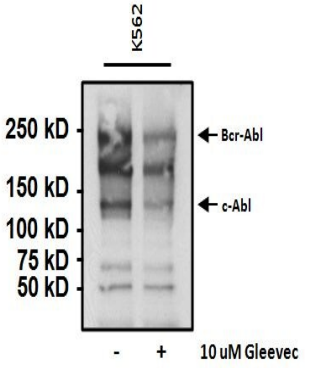
Phosphotyrosine Antibody (MA4-003) in ICC/IF

Immunofluorescent analysis of Phosphotyrosine (p-Tyr, green) in HeLa cells. Cells were probed with a p-Tyr monoclonal antibody (Product # MA4-003) followed by a fluorescently-conjugated anti-mouse IgG secondary antibody.



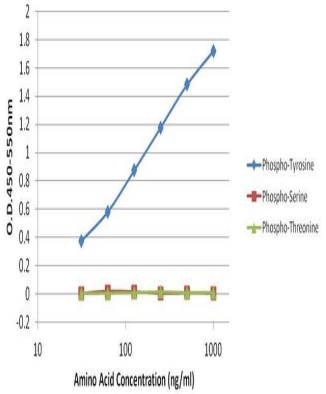
Phosphotyrosine Antibody (MA4-003) in WB

Western blot analysis of tyrosine phosphorylated c-Abl and Bcr-Abl was performed following immunoprecipitation of K562 cell lysates using a c-Abl antibody, from untreated cells (left lane) or cells treated with 10uM of the tyrosine kinase inhibitor Gleevec (right lane) for 24 hours. The captured immune complexes were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a Phosphotyrosine monoclonal antibody (Product # MA4-003) at a dilution of 1:250 overnight rotating at 4°C, washed in TBST, and probed with a goat anti-mouse IgG-HRP secondary antibody (Product # 31430) at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).



Phosphotyrosine Antibody (MA4-003) in IP

Immunoprecipitation of Phosphotyrosine (p-Tyr) was performed on K562 cell lysates from untreated cells (left lane) or from cells treated with 10uM of the tyrosine kinase inhibitor Gleevec (right lane) for 24 hours. Antigen-antibody complexes were formed by incubating 500 µg of whole cell lysate with 2 µg of a p-Tyr monoclonal antibody (Product # MA4-003) overnight on a rocking platform at 4°C. The immune complexes were captured on 50 µL Protein A/G Agarose (Product # 20421), washed extensively, and eluted with Lane Marker Reducing Sample Buffer (Product # 39000). Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a c-Abl polyclonal antibody at a dilution of 1:1000 overnight rotating at 4°C, washed in TBST, and probed with goat anti-rabbit IgG-HRP secondary antibody (Product # 31460) at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).



Phosphotyrosine Antibody (MA4-003) in ELISA

Direct ELISA analysis of Phosphotyrosine was performed by coating wells of a 96-well plate with 100 µL per well of Phosphotyrosine, Phosphoserine or Phosphothreonine diluted in carbonate/bicarbonate buffer (Product # 28382) at a concentration of 5 µg/mL overnight at 4C. Wells of the plate were washed, blocked with StartingBlock blocking buffer (Product # 37538), and incubated with 100 µL per well of a mouse anti-phosphotyrosine monoclonal antibody (Product # MA4-003) at a starting concentration of 1 µg/mL and serially diluting 2-fold to a concentration of 31 ng/mL for 30 minutes at 37C. The plate was washed, then incubated with 100 µL per well of an HRP-conjugated goat anti-mouse IgG secondary antibody (Product # 31430) at a dilution of 1:8000 for 30 minutes at 37C. Detection was performed using 1-Step Ultra TMB substrate (Product # 34028) for 5 minutes at room temperature in the dark. The reaction was stopped with 0.16M sulfuric acid, and absorbances were read on a spectrophotometer at 450-550 nm.

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PubMed References For Phosphotyrosine Monoclonal Antibody (IG2)

1 Western Blot References

Species / Dilution	Summary
Chemical / Not Cited	MA4-003 was used in western blot to investigate the role of JNK and tumor necrosis factor-alpha in free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. The Journal of biological chemistry (Oct 2005; 280: 35361) **"JNK and tumor necrosis factor-alpha mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes."** Author(s):Nguyen MT,Satoh H,Favelyukis S,Babendure JL,Imamura T,Sbodio JI,Zalevsky J,Dahiyat BI,Chi NW,Olefsky JM PubMed Article URL:http://dx.doi.org/10.1074/jbc.M504611200

1 Immunoprecipitation References

Species / Dilution	Summary
Chemical / Not Cited	MA4-003 was used in immunoprecipitation to investigate the important role of ABL protein possessing constitutive protein-tyrosine kinase activity in chronic myelogenous leukemia Proceedings of the National Academy of Sciences of the United States of America (Jul 1987; 84: 4408) **"Cell lines and peripheral blood leukocytes derived from individuals with chronic myelogenous leukemia display virtually identical proteins phosphorylated on tyrosine residues."** Author(s):Huhn RD,Posner MR,Rayter SI,Foulkes JG,Frackelton AR PubMed Article URL:http://dx.doi.org/10.1073/pnas.84.13.4408

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