AP-Conjugated Monoclonal Anti-Phospho-Tyrosine Antibody

ORDERING INFORMATION

Catalog Number: APM1676

Clone: 179003

Lot Number: JLA01

Size: 100 µL (sufficient for 100 mL of blotting solution)

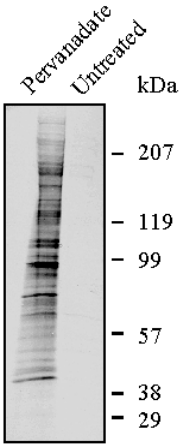
Storage: 2 - 8° C

Specificity: proteins containing phospho-tyrosine

Immunogen: phospho-tyrosine:KLH

Ig Type: mouse IgG_1

Applications: Western blot
ELISA



Detection of tyrosine-phosphorylated proteins with APM1676. Lysates from A431 cells either untreated or incubated with 50 µM pervanadate for 15 minutes were resolved by SDS-PAGE, transferred to Immobilon-P membrane and detected with AP-conjugated monoclonal anti-phospho-tyrosine (1000x dilution), as described in *Protocols for Immunoblotting and ELISA*.

Preparation
This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with short peptides containing phospho-tyrosine coupled to KLH. The IgG fraction of the hybridoma culture supernatant was purified by protein G chromatography and then conjugated with calf intestinal alkaline phosphatase (AP).

Formulation
Supplied as a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with stabilizers and preservatives.

Storage
Neat samples can be stored at 2° to 8° C for at least 3 months without detectable loss of activity. **Do not freeze.**

Specificity
The antibody detects endogenous proteins containing phosphorylated tyrosine residues. ELISA and Western blot analyses using pervanadate-treated cell lysates indicate that clone 179003 binds phospho-tyrosine in a broad manner largely independent of the surrounding amino acid sequence. The antibody does not cross-react with proteins or peptides containing phosphorylated serine or threonine residues.

Applications
For Western blot analysis, an antibody dilution of 1:1,000 is recommended.

For ELISA, the recommended working dilution is 1:5,000.
Optimal dilutions should be determined by each laboratory for each application.

Protocols for Immunoblotting and ELISA:

Western blotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% BSA in Blotting Buffer	5% BSA in Blotting Buffer
0.15 M NaCl	Adjust pH to 7.4	Adjust pH to 7.4
0.1% Tween® 20		

1. Transfer electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2 - 8° C in Antibody Solution containing a 1000-fold dilution of AP-conjugated mouse anti-phospho-tyrosine.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Detect with NBT/BCIP substrate development reagent (Pierce).

Samples for Western blotting: Protein preparations are added to an equal volume of 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue), heated in a boiling water bath for 5 minutes, and resolved by SDS-PAGE.

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12/22/03

ELISA

Cell Lysis

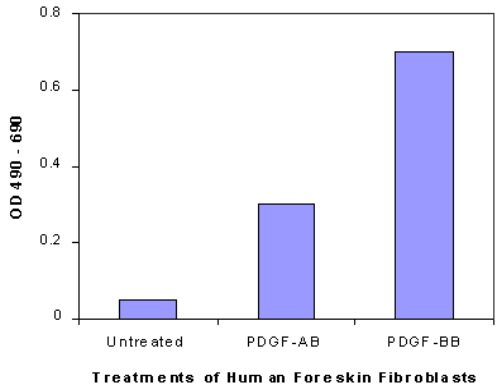
1. Rinse cells three times with cold PBS.
2. Solubilize 1 x 10^6 - 5 x 10^6 cells per mL of cold Cell Lysis Buffer (20 mM Tris, pH 8.0, 0.15 M NaCl, 2 mM EDTA, 1% NP-40, 10% glycerol, 1 mM Na_3VO_4, 0.25 mM PMSF, 1 μg/mL aprotinin, 1 μg/mL chymostatin, 1 μg/mL leupeptin). The lysate mixtures are rocked at 4° C for 30 - 60 minutes to extract proteins.
3. Centrifuge cell lysates at 3,000 x g for 5 minutes to remove insoluble material.

Plate Preparation

1. Dilute the capture antibody to a working concentration of 1 - 10 μg/mL in PBS. Immediately coat a 96 well microplate (Costar) with 100 μL per well of the diluted capture antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash 3 times with 300 μL of Wash Buffer (0.05% Tween® 20 in PBS, pH 7.2 - 7.4).
3. Block plates by adding 300 μL of Block Buffer (1% BSA, 5% Sucrose, 0.05% NaN_3, in PBS, pH 7.2 - 7.4). Incubate at room temperature for 1 - 2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 μL of diluted sample in Cell Lysis Buffer per well. Incubate 2 hours at room temperature with shaking.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Dilute the AP-conjugated mouse anti-phospho-tyrosine detection antibody 5000-fold in PBS containing 1% BSA, pH 7.2 - 7.4. Add 100 μL of the diluted detection antibody to each well and incubate 2 hours at room temperature with shaking.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 50 μL of AMPAK® Substrate Solution (DAKO) containing NADPH to each well. Incubate for 60 minutes at room temperature with shaking.
6. Add 50 μL of AMPAK® Amplifier Solution (DAKO) containing alcohol dehydrogenase, diaphorase, and INT-Violet to each well. Incubate for 5 - 30 minutes at room temperature.
7. Add 50 μL of Stop Solution (2 N H_2SO_4) to each well. Gently tap the plate to ensure thorough mixing.
8. Determine the OD of each well within 30 minutes, using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the reading at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.



Phospho-PDGF Rβ ELISA using APM1676 as detection antibody.
Human Foreskin Fibroblasts were untreated or treated with 100 ng/mL human PDGF-AB (R&D Systems, Catalog # 222-AB) or PDGF-BB (R&D Systems, Catalog # 220-BB), respectively, for 5 minutes at 37° C in serum-free medium. Activation of PDGF Rβ was assessed by ELISA using an anti-human PDGF Rβ as the capture antibody and APM1676 as the detection antibody.