

## HRP-Conjugated Monoclonal Anti-Phospho-Tyrosine Antibody

### ORDERING INFORMATION

**Catalog Number:** HAM1676

**Clone:** 179003

**Lot Numbers:** JLB22  
JLB23

**Size:** 100 µL (sufficient for 500 mL  
of blotting solution)

**Storage:** 2 - 8 °C

**Specificity:** proteins containing  
Phospho-Tyrosine

**Immunogen:** Phospho-Tyrosine:KLH

**Ig Type:** mouse IgG<sub>1</sub>

**Recommended Applications:**  
Western blot  
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 horseradish peroxidase (HRP).

### 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 - 8 °C for at least 3 months without detectable loss of activity. **Do not freeze.**

### Specificity

This antibody detects endogenous proteins containing phosphorylated tyrosine residues. ELISA and 2D Western blot analyses using pervanadate-treated cell lysates indicate that this antibody 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

**Western blot** - An antibody dilution of 1:5,000 is recommended.

**ELISA** - An antibody dilution of 1:5,000 is recommended.

**Optimal dilutions should be determined by each laboratory for each application.**

### Protocols for Immunoblotting and ELISA

#### Western blotting

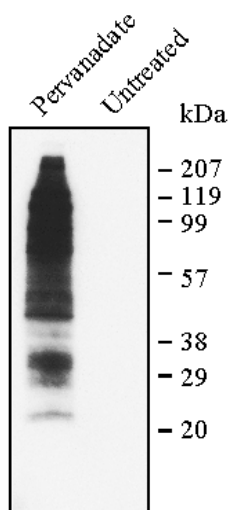
**Blotting Buffer**  
25 mM Tris, pH 7.4  
0.15 M NaCl  
0.1% Tween® 20

**Blocking Solution**  
5% BSA in Blotting Buffer  
Adjust pH to 7.4

**Antibody Solution**  
5% BSA in Blotting Buffer  
Adjust pH to 7.4

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 1:5,000 dilution of HRP-conjugated 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 Chemiluminescent Detection Substrate.

**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.



**Detection of tyrosine-phosphorylated proteins with HAM1676.** 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 HRP-conjugated monoclonal anti-Phospho-Tyrosine (1:5,000 dilution), as described in *Protocols for Immunoblotting and ELISA*. A one minute exposure to film is shown.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

**R&D Systems, Inc.**  
**1-800-343-7475**

## ELISA

### Cell Lysis

1. Rinse cells three times with cold PBS.
2. Solubilize  $1 - 5 \times 10^6$  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  $\text{Na}_3\text{VO}_4$ , 0.25 mM PMSF, 1  $\mu\text{g/mL}$  aprotinin, 1  $\mu\text{g/mL}$  chymostatin, 1  $\mu\text{g/mL}$  leupeptin). Rock the lysate mixtures at 4 °C for 30 - 60 minutes to extract proteins.
3. Centrifuge cell lysates at  $3,000 \times g$  for 5 minutes to remove insoluble material.

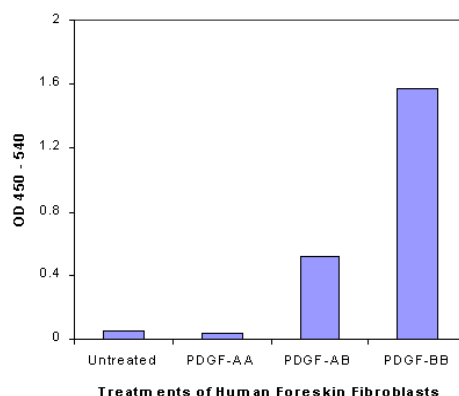
### Plate Preparation

1. Dilute the capture antibody to a working concentration of 1 - 10  $\mu\text{g/mL}$  in PBS. Immediately coat a 96 well microplate (Costar) with 100  $\mu\text{L}$  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  $\mu\text{L}$  of Wash Buffer (0.05% Tween® 20 in PBS, pH 7.2 - 7.4).
3. Block plates by adding 300  $\mu\text{L}$  of Block Buffer (1% BSA, 5% Sucrose, 0.05%  $\text{NaN}_3$  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  $\mu\text{L}$  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 HRP-conjugated anti-Phospho-Tyrosine detection antibody 5,000-fold in PBS containing 1% BSA, pH 7.2 - 7.4. Add 100  $\mu\text{L}$  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 100  $\mu\text{L}$  of Substrate Solution (R&D Systems, Catalog # DY999) to each well. Incubate for 5 - 20 minutes at room temperature.
6. Add 50  $\mu\text{L}$  of Stop Solution (2 N  $\text{H}_2\text{SO}_4$ ) to each well. Gently tap the plate to ensure thorough mixing.

Determine the OD of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the reading at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.



#### Phospho-PDGF R $\beta$ ELISA using HAM1676 as detection antibody.

Human Foreskin Fibroblasts were untreated or treated with 100 ng/mL human PDGF-AA (R&D Systems, Catalog # 221-AA), 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 R $\beta$  was assessed by ELISA using an anti-human PDGF R $\beta$  as the capture antibody and HAM1676 as the detection antibody.