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AM00124PU-N Monoclonal Antibody to Phosphotyrosine (incl. pos. control) - Purified

Quantity:	0.1 mg
Background:	Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.
Host / Isotype:	Mouse / IgG1
Recommended Isotype Controls:	SM10P (for use in human samples), SM20P (for use in rat samples), AM03095PU-N
Clone:	2C8
Immunogen:	Synthetic phosphopeptide conjugated to KLH
Format:	**State:** Lyophilized purified Ig **Purification:** Size exclusion chromatography **Buffer System:** 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose **Reconstitution:** Restore with 1 ml H2O (15 min, RT).
Applications:	Western Blot: 0.5 µg/ml for HRPO/ECL detection. Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer. ELISA: 0.1 µg/ml. Immunoprecipitation: 1 - 10 µg per 106 pervanadate-treated A431 cells. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	Mab PTYR-2C8 recognizes a broad range of tyrosine--phosphorylated proteins in crude cell extracts and may therefore be particularly well-suited for the detection/screening of tyrosine phosphorylated proteins. **Species:** Human, Mouse, Rat, Dog. Other species not tested.
Add. Information:	Includes positive control (see protocols).
Storage:	Store lyophilized (preferably in a desiccator) at -20°C and reconstituted (aliquote and freeze in liquid nitrogen) at -20°C to -80°C. Avoid repeated freezing and thawing. Thaw aliquots at 37°C. Thawed aliquots may be stored at 2-8°C up to 3 months. Shelf life: one year from despatch.

Protocols:

Phosphotyrosine MW standard
Size: 20 Blots
Formulation: Phosphotyrosine modified standard proteins lyophilized from PBS / 0.1 % SDS / PEG / Sucrose / Malachitgreen.
The following standard proteins were modified with phosphotyrosine: galactosidase (116 kD), phosphorylase A (98 kD), BSA (67 kD), ovalbumin (46 kD), carbonic anhydrase (32 kD), and soybean trypsin inhibitor (24 kD).
Storage: Reconstitute by addition of 200 µl H2O. After complete solubilization add 200 µl 2x SDS-PAGE sample buffer, mix and incubate at 90°C for 5 min.
Application: The phosphptyrosine molecular weight marker is recommended for immunoblot applications. Use 20 µl of the phosphotyrosine molecular weight marker per lane (mini gel).
The individual proteins of the marker are recognized by the following commercially available clones: 2C8, 1F9, 3B12, 9F1, 9H8, 16F4.

Pictures:

Phosphotyrosine Detection Lysates of pervanadate-treated A431 cells were probed with LANE1: mab 2A5 (IgG), 1µg/ml LANE2: mab 2C8 (IgG), 1µg/ml LANE3: mab 3B12 (IgG), 1µg/ml LANE4: mab 9H8 (IgG), 1µg/ml LANE5: mab 16F4 (IgG), 1µg/ml LANE6: mab 9F1 (IgG), 1µg/ml