V 3.0

Anti-Phosphotyrosine-HRP Mouse MAb

Cat. # APY03-HRP

Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 100 µl when reconstituted
Validated applications:	WB
Species reactivity:	All
Host/Isotype:	Mouse/IgG2b
Clone:	27B10.4

Background Information
Tyrosine phosphorylation, a reversible process, is one of the most frequent post-translational modifications of proteins and is crucial in mediating signal transduction in eukaryotic cells after exposure to cytokines and growth factors (1). Anti-phosphotyrosine antibodies have been important tools in studying the level of tyrosine phosphorylation of proteins in different cellular models. They have also played an important role in enriching phosphotyrosine peptides from trypsin-digested cell lysates. As a result a large number of phosphopeptides have been identified under various physiological and pathological conditions with mass spectrometry technologies (2-3).

Material
APY03-HRP anti-phosphotyrosine antibody is a mouse monoclonal antibody that recognizes proteins post-translationally modified by phosphorylation of tyrosine residues. APY03 was raised against a proprietary mixture of phosphotyrosine peptides conjugated to KLH. It has been shown to recognize a wide range of tyrosine phosphorylated proteins in NIH3T3 cells treated with H_2O_2/vanadate (Figure 1) and can detect 10 ng of phosphotyrosine-labeled bovine serum albumin (see Certificate of Analysis [COA]). APY03 is purified by protein G affinity chromatography and is supplied as a lyophilized white powder. Each Lot of antibody is quality controlled to provide a high batch to batch consistency. The Lot specific µg per tube can be found in the Lot specific COA documents at www.cytoskeleton.com. APY03-HRP shows high specificity to phosphotyrosine proteins and does not cross-react with phosphoserine/threonine proteins a western blot assay (Figures 1).

Storage and Reconstitution
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube.

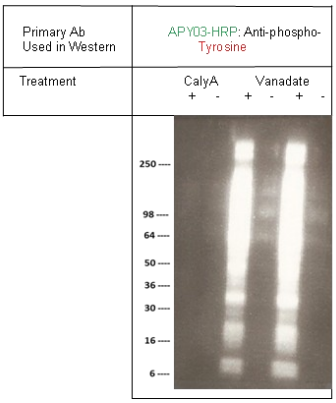
Reconstitute each tube first in 50µl of Milli-Q water and then add 50µl of glycerol. Alternatively resuspend in 100 µl of 50% glycerol (room temperature). We do not recommend using 50% glycerol at 4°C as this can cause the lyophilized antibody to stick to the pipet tip during resuspension. Store reconstituted antibody at –20°C. Final buffer composition is 200 mM PIPES, 50% glycerol, 1% sucrose,1% dextran and 10mg/ml BSA.

When stored and reconstituted as described, the product is stable for 6 months at -20°C. **NOTE: Sodium azide is an irreversible inhibitor of HRP. Do not add sodium azide to APY03-HRP antibody**.

Applications

Western Blot (WB) Applications
Use as indicated in method at 1:6000 dilution, sufficient for 600 ml of working strength Ab.

Western Blot Method:
1. Run protein samples and control samples on SDS-PAGE.
2. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, 5% methanol) for 15 min at room temperature prior to electro-blotting.
3. Transfer the protein to a PVDF membrane overnight at 20V at 4°C.
4. Wash the membrane once with TBST for 10 minutes (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Block the membrane surface with 5% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
6. Incubate the membrane with a 1:6000 dilution of APY03-HRP antibody diluted in TBST/1.5% milk for 1h at room temperature or overnight at 4°C with constant agitation.
7. Wash the membrane 6 times in TBST for 10 min each.
8. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).



Legend: A431 cells were either treated (**+**) or untreated (**-**) with Calyculin A (CalyA: a serine/threonine phosphatase inhibitor, 50nM for 1 hour). NIH3T3 cells were either treated or untreated with H_2O_2-activated sodium orthovanadate (Vanadate: a specific tyrosine phosphatase inhibitor, 100 µM for 10 minutes). 10 µg of each lysate was resolved in SDS-PAGE and proteins were transferred to PVDF membrane. APY03-HRP (1:6000) was used to detect tyrosine phosphorylated proteins. Western blot was developed with SuperSignal West Dura chemiluminescent reagent (Thermo Scientific) and exposure time was 10 seconds. As shown in Fig.1, a wide range of tyrosine phosphorylated proteins were detected in NIH3T3 cells treated with orthovanadate but not in Calyculin A treated A431 cells.

Figure 1: Western Blot: Demonstration of APY03-HRP phosphotyrosine specificity

References
1. Machida, K. et al. (2003) Profiling the global tyrosine phosphorylation state. Mol. Cell. Proteomics 2, 215-233
2. Blagoev, B. et al. (2004) Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics. Nat. Biotechnol. 22, 1139-1145
3. Schmelzle, K. et al. (2006) Temporal dynamics of tyrosine phosphorylation in insulin signaling. Diabetes 55, 2171-2179

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