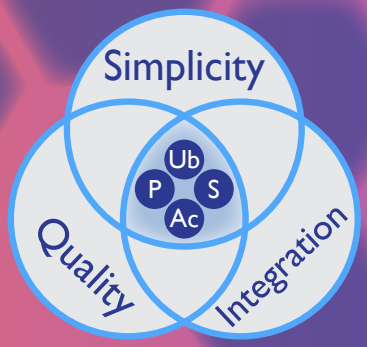


Discover New Tyrosine Phosphorylation Targets

Superior Endogenous pY Enrichment and Detection

The Protein Experts

Cytoskeleton, Inc.

Signal-Seeker Toolkits
by ***Cytoskeleton, Inc.***

Signal-Seeker™ Phosphorylation Tools

About Tyrosine Phosphorylation

The post-translational modification (PTM) of tyrosine residues by the reversible addition of a phosphate group is a powerful signaling switch in a wide range of cellular events (1). Many growth factors act through receptor tyrosine kinases and subsequent phospho-tyrosine/serine/threonine cascades to control cell proliferation, migration, and adhesion (2). The deregulation of tyrosine phosphorylation is known to underlie many diseases including cancers and many of the 90 human tyrosine kinases are targets for the development of anti-cancer therapeutics (3). Some well known tyrosine kinase inhibitors include Gleevec™ approved for the treatment of chronic myeloid leukemia (CML) and IressaTM and TarcevaTM for the treatment of non small cell lung cancer (NSCLC) (3).

Because of the critical importance of tyrosine phosphorylation in normal and aberrant cell functions, there is great interest in identifying the phosphotyrosine profile of single proteins, protein pathways and whole cells under a variety of conditions and dynamic states. Phosphotyrosine antibodies are a powerful tool in helping elucidate the role of this PTM in cellular functions.

Superior Tyrosine Phosphorylated Protein Enrichment for Engodenous pY Investigation

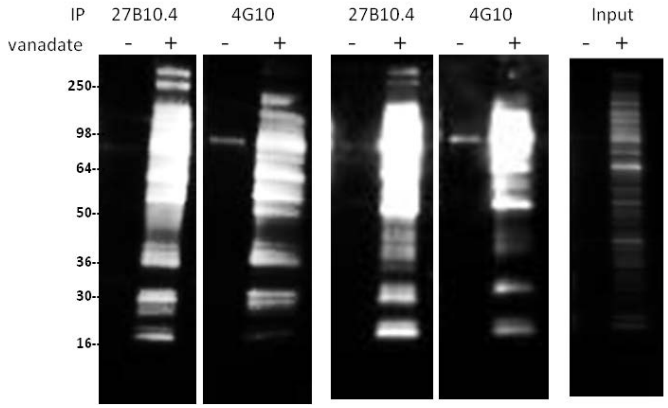


Figure 1 Legend: NIH3T3 cells were either treated (+) or untreated (-) with pervanadate (100 µM for 10min). 200 µg of lysate per reaction was used for immunoprecipitation of tyrosine-phosphorylated proteins. 27B10.4 or 4G10 (5 µl at 1 µg/µl) was first bound to protein G beads and then incubated with cell lysate. Western blots of immunoprecipitated proteins were developed using 27B10.4 at 1:500 dilution and CleanBlot (Thermo Scientific, #21230) at 1:1000 dilution) as secondary antibody. Input represents the signal from 5% of pervanadate treated or untreated NIH3T3 lysate.

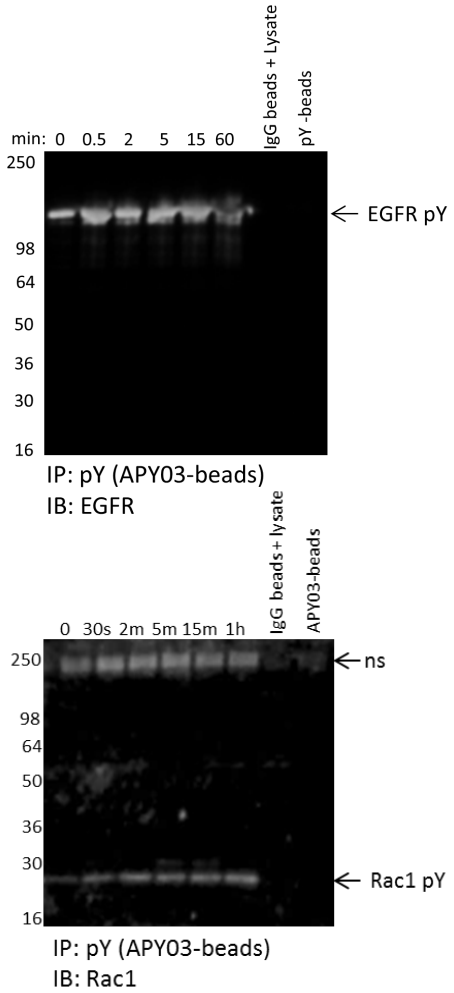


Figure 2 Legend: Serum-restricted A431 cells were stimulated with EGF for the given time period. APY03-beads (phosphotyrosine affinity beads) were used to immunoprecipitate tyrosine-phosphorylated proteins. Samples were separated by SDS-PAGE and transferred to PVDF. Western blot was performed with an EGFR or Rac1 antibody to detect tyrosine phosphorylated EGFR or Rac1 respectively.

Phosphotyrosine Products

Description	Amount	Item #
Signal-Seeker™ Phosphotyrosine Detection Kit	30 assays	BK160
Signal-Seeker™ Phosphotyrosine Detection Kit	10 assays	BK160-S
Phosphotyrosine Affinity Beads	40 assays	APY03-beads
Mouse IgG Control Beads	10 assays	CIG01-beads
Phosphotyrosine Mouse Antibody (27B10)	2 x 100 ml	APY03
Phosphotyrosine Mouse Antibody-HRP labeled	1 x 100 ml	APY03-HRP

Phosphotyrosine Tools For Your Research Needs

Applications

Application	Product	Validation Data
Western Blot	Phosphotyrosine Mouse Antibody-HRP labeled, Cat. # APY03-HRP	Yes
	Phosphotyrosine Mouse Antibody (27B10), Cat. # APY03	Yes
Immunofluoresence	Phosphotyrosine Mouse Antibody (27B10), Cat. # APY03	Yes
Immunoprecipitation	Signal-Seeker™ Phosphotyrosine Detection Kit, Cat. # BK160	Yes
	Signal-Seeker™ Phosphotyrosine Detection Kit, Cat. # BK160-S	Yes
	Phosphotyrosine Affinity Beads, Cat. # APY03-beads	Yes
	Phosphotyrosine Mouse Antibody (27B10), Cat. # APY03	Yes

*Recommended products for each application are highlighted in **blue**

Sensitive IF and IHC phosphotyrosine detection

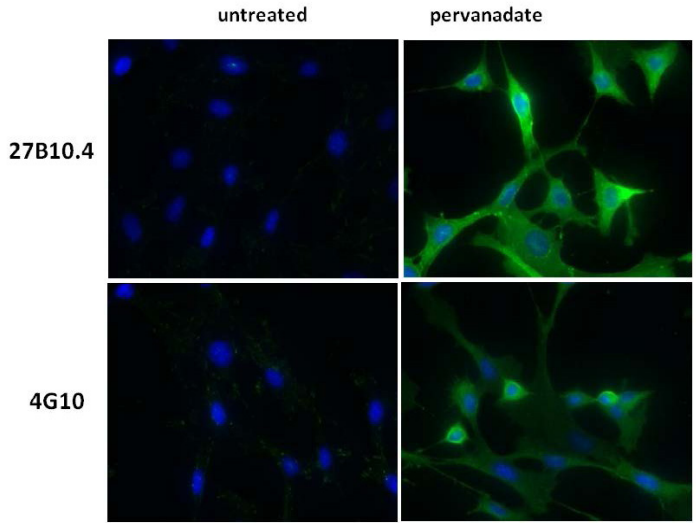


Figure 3 Legend: NIH3T3, untreated or treated with pervanadate (100 µM for 10 min), were stained with a 1:1000 dilution of phosphotyrosine antibody as described in the APY03 datasheet (see www.cytoskeleton.com). Phosphotyrosine and nuclei were visualized in green fluorescence and blue DAPI staining, respectively. Top images are from cells probed with 27B10.4, bottom set of images are from cells probed with 4G10.

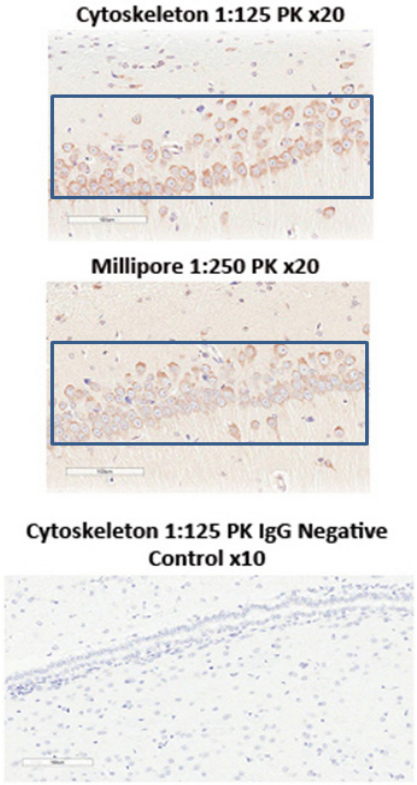


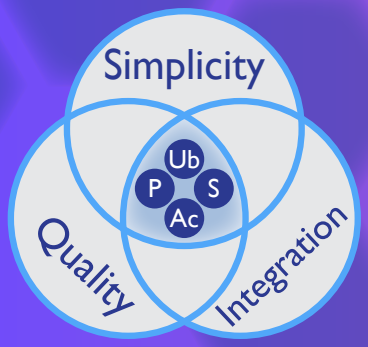
Figure 4 Legend: Anti-pan-phosphotyrosine immunohistochemistry in rat cortical tissue. Cortical brain sections were prepared and processed as described in material and methods (see www.cytoskeleton.com). Following Proteinase K (PK) antigen retrieval, each primary antibody (clone 27B10.4, Cytoskeleton or clone 4G10, Millipore) was used at what was determined by Wax-it to be the optimal immunohistochemical dilution and imaged at 20X. The bottom image is the IgG negative control for the 27B10.4 antibody.

References

1. Lim W. and Pawson T. 2010. Phosphotyrosine signaling: evolving anew cellular communication system. Cell 142:661-667.

2. Wagner MJ et al. 2013. Molecular mechanisms of SH2– and PTB–domain-containing proteins in receptor tyrosine kinase signaling. Cold Spring Harb. Perspect. Biol. 5:a008987.

3. Hunter T. 2009. Tyrosine phosphorylation: thirty years and counting. Curr. Opin. Cell Biol. 21:140-146.



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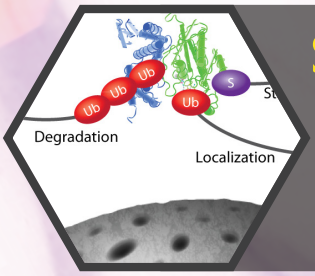
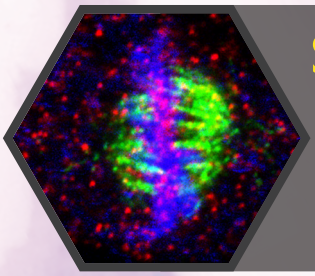


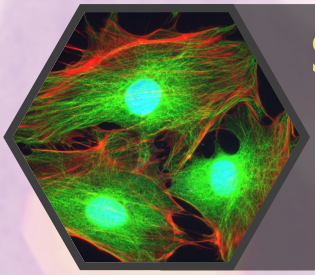
Signal-Seeker™ Ubiquitin Tools

- Mono-/Poly-Ubiquitination Detection Kit
- Mono-/Poly-Ubiquitination Affinity Beads
- Ubiquitin Antibody-HRP labeled



Signal-Seeker™ SUMOylation 2/3 Tools

- SUMO 2/3 Detection Kit
- SUMO 2/3 Affinity Beads
- SUMO 2/3 IF and HRP labeled Antibodies



Signal-Seeker™ Acetyl-lysine Tools

- Acetyl-lysine Detection Kit
- Acetyl-lysine Affinity Beads
- Acetyl-lysine IF and HRP labeled Antibodies

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