

## Phosphorylated Tyrosine Molecular Weight Standards

Catalog # 12-256

Lot # 15471

**Background:** Tyrosine kinases have been shown to play an important role in signal transduction cascades. Transmembrane receptors tend to fall into two basic groups: (1) receptors which contain an intracellular protein tyrosine kinase (PTK) domain which becomes activated after binding of its ligand and; (2) receptors which do not contain an intracellular PTK domain but which must interact with and activate cytoplasmic tyrosine kinases after binding of its ligand. Many growth factor receptors contain a PTK domain while many cytokine receptors do not contain a PTK domain and require cytoplasmic tyrosine kinases (for example, Jaks, Syk, or Faks) to exert their intracellular signalling. The phosphorylated tyrosine molecular standards can be used to characterize many tyrosine kinases.

**Formulation:** Phosphorylated tyrosine MW standards, which were prepared from soybean trypsin inhibitor, ovalbumin, and bovine serum albumin (BSA), in **150ml** of phosphate-buffered saline, pH 7.5.

**Molecular Weight Range:** Recommended for a range from 28kDa to 85kDa.

**Blot Applications:** 150 immunoblots.

**Physical Form:** Frozen liquid.

**Storage and Shelf Life:** 1 year at -20°C. Avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

### References:

- Ohshima, H., *et al.*, Ed. Chem. Tox. **28**: 647-652, 1990.  
Ischiropoulos, H., *et al.*, Arch. Biochem. Biophys. **298**: 431-437, 1992.  
Kono, S., *et al.*, Biochem. Biophys. Res. Comm. **190**: 283-288, 1993.

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN HUMANS.**

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### **Quality Control Testing and Research Applications**

Western Immunoblot Analysis: 1 $\mu$ l of this lot of standards was detected in a western immunoblot using a primary anti-phosphotyrosine antibody (Upstate Biotechnology Inc. Cat. # 05-321 at 1 $\mu$ g/ml) and enhanced chemiluminescence detection. Three major bands were observed at  $\approx$  28kDa,  $\approx$  45kDa, and  $\approx$  85kDa on this blot.

### **Western Immunoblot Protocol**

1. Dilute the phosphorylated tyrosine molecular weight standard 1:10 with 1X Laemmli reducing sample buffer, i.e. 1 $\mu$ l standards with 9 $\mu$ l sample buffer.
2. Load 10 $\mu$ l of diluted phosphorylated tyrosine molecular weight standards, along with your samples, on a polyacrylamide gel. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
3. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25 $^{\circ}$ C with constant agitation.
4. Incubate the nitrocellulose in the phosphotyrosine antibody (1 $\mu$ g/ml) diluted in freshly prepared PBS-MLK overnight with agitation at 4 $^{\circ}$ C.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose in the secondary reagent of choice (1:3000 dilution) in PBS-MLK for 1.5 hours at 20-25 $^{\circ}$ C with agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice: enhanced chemiluminescence or enzygraphic web is recommended.