

## Phosphotyrosine Molecular Weight Standards

Catalog # 12-256

Lot # 16855

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

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

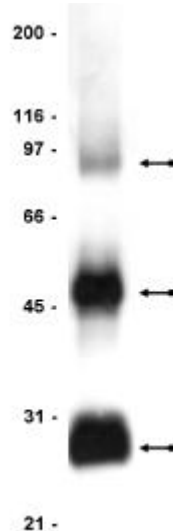
**Blot Applications:** 150 immunoblots.

**Storage and Stability:** Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

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

### Quality Control Testing

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



#### Immunoblot Analysis

Phosphotyrosine Molecular Weight Standards were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Phosphotyrosine (1 $\mu$ g/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

**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 signaling. The phosphorylated tyrosine molecular standards can be used to characterize many tyrosine kinases.

#### 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.

### Immunoblot Protocol

1. **Dilute the phosphotyrosine molecular weight standards 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°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°C.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse IgG linked to horseradish peroxidase, 1:1000 dilution) in PBS-MLK for 1.5 hours at 20-25°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 enzymatic web is recommended.