

## Certificate of Analysis

### Phosphotyrosine Molecular Weight Standards

Catalog # 12-256

Lot # 21150

**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. Frozen solution.

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

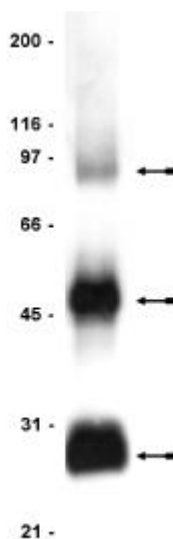
**Blot Applications:** 150 immunoblots.

**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 proteins were detected at ~28kDa, ~45kDa, and ~85kDa on this blot.



#### Immunoblot Analysis

Representative blot from a previous lot. 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.

#### 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, Catalog # 12-349, 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.