

## Reference

1. Schägger, H., and von Jagow, G., *Anal. Biochem.*, **166**, 368-379 (1979).

## Ordering Information

| Catalog Number                    | Product Description  |
|-----------------------------------|--|
| <b>Premixed Buffers</b>           |  |
| 161-0739                          | <b>Tricine Sample Buffer</b> , 30 ml                       |
| 161-0744                          | <b>10x Tris/Tricine/SDS</b> , 1 L                          |
| <b>Prestained Standards</b>       |  |
| 161-0325                          | <b>Kaleidoscope Polypeptide Standards</b> , 500 $\mu$ l    |
| 161-0324                          | <b>Kaleidoscope Prestained Standards</b> , 500 $\mu$ l     |
| 161-0305                          | <b>Prestained SDS-PAGE Standards</b> , Low, 500 $\mu$ l    |
| 161-0309                          | <b>Prestained SDS-PAGE Standards</b> , High, 500 $\mu$ l   |
| 161-0318                          | <b>Prestained SDS-PAGE Standards</b> , Broad, 500 $\mu$ l  |
| <b>Molecular Weight Standards</b> |  |
| 161-0326                          | <b>Polypeptide SDS-PAGE Standards</b> , 200 $\mu$ l        |
| 161-0304                          | <b>SDS-PAGE Standards</b> , Low, 200 $\mu$ l               |
| 161-0303                          | <b>SDS-PAGE Standards</b> , High, 200 $\mu$ l              |
| 161-0317                          | <b>SDS-PAGE Standards</b> , Broad, 200 $\mu$ l             |
| 161-0314                          | <b>Silver Stain SDS-PAGE Standards</b> , Low, 200 $\mu$ l  |
| 161-0315                          | <b>Silver Stain SDS-PAGE Standards</b> , High, 200 $\mu$ l |

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4006046 Rev B



# Polypeptide SDS-PAGE Molecular Weight Standards

**Catalog Number**  
**161-0326**

**Product shipped at room temperature.**  
**Store at -20 °C upon arrival.**



**BIO-RAD**

# Specifications

|                                    |  |
|------------------------------------|--|
| <b>Contents</b>                    | Approximately 900 µg of each protein blended to give bands of equal intensity on SDS polyacrylamide gels run according to Schägger and von Jagow <sup>1</sup> and stained with Coomassie blue G-250 stain. |
| <b>Storage buffer</b>              | 40% glycerol, 100 mM Tris-HCl, 4 mM EDTA, 3 mM NaN <sub>3</sub><br>pH 8.5  |
| <b>Volume</b>                      | 200 µl concentrated solution   |
| <b>Storage</b>                     | -20 °C   |
| <b>Shipping conditions</b>         | Room temperature   |
| <b>Shelf life</b>                  | 1 year at -20 °C   |
| <b>Applications per vial</b>       | 400  |
| <b>Recommended gel percentage*</b> | 16.5% Tris-Tricine<br>10-20% Tris-Tricine  |

**\*Note:** These standards can be run on other percentage gels, but all proteins may not be visible. Lower percentage gels or Tris-glycine buffer systems may cause the low molecular weight proteins to migrate with or in front of the dye front.

## Protein Molecular Weights (daltons)

| <b>Protein</b>            | <b>Molecular Weight</b> |
|---------------------------|-------------------------|
| Triosephosphate isomerase | 26,625                  |
| Myoglobin                 | 16,950                  |
| α-Lactalbumin             | 14,437                  |
| Aprotinin                 | 6,512                   |
| Insulin b chain, oxidized | 3,496                   |
| Bacitracin                | 1,423                   |

## Protocol

Dilute standards 1:20 in Tris-Tricine Sample Buffer.\* Heat for 5 minutes at 95 °C. Cool and load 10 µl/well for full length gels (16-20 cm) or 5 µl/well for mini gels.

### Tris-Tricine Sample Buffer

|                       |         |
|-----------------------|---------|
| Deionized water       | 4.0 ml  |
| 0.5M Tris-HCl, pH 6.8 | 2.0 ml  |
| Glycerol              | 2.4 ml  |
| 10% SDS               | 1.0 ml  |
| β-mercaptoethanol     | 0.2 ml  |
| 0.5% Coomassie G-250  | 0.4 ml  |
|                       | 10.0 ml |

Use of Sample Buffer with insufficient or old β-mercaptoethanol may result in doublets or diffuse bands.

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### Coomassie Blue G-250 Staining Solution

|                      |        |
|----------------------|--------|
| Acetic acid          | 100 ml |
| Coomassie Blue G-250 | 0.25 g |
| Deionized water      | 900 ml |

**Note:** Use stain only once.

### Coomassie Blue G-250 Destaining Solution

|                 |        |
|-----------------|--------|
| Acetic acid     | 100 ml |
| Deionized water | 900 ml |

## Protein References

| Protein                           | Reference  |
|-----------------------------------|--|
| Rabbit triosephosphate isomerase  | Corran, P. H. and Waley, S. G., <i>Biochem. J.</i> , <b>139</b> , 1 (1974).                  |
| Equine myoglobin                  | Black, J. A. and Leaf, G., <i>Biochem. J.</i> , <b>96</b> , 693 (1965).                      |
| Bovine α-Lactalbumin              | Brew, K., Vanaman, T. C., and Hill, R. L., <i>J. Biol. Chem.</i> , <b>242</b> , 16 (1967).   |
| Bovine aprotinin                  | Kassell, B. and Laskowski, M., <i>Biochem. Biophys. Res. Comm.</i> , <b>20</b> , 463 (1965). |
| Bovine insulin, b chain, oxidized | Porter, R. R., <i>Biochem.</i> , <b>53</b> , 320 (1953).                                     |
| Bacitracin                        | Merck Index, 11, 948.  |

## Gel Staining

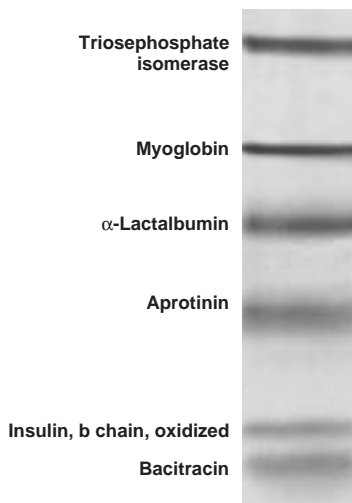
Approximately 0.5 µg of protein per band is needed for detection when gels are stained with Coomassie Blue G-250. Place the gels in polypeptide fixative solution for 30 minutes. Stain in Coomassie blue G-250 Staining Solution for 1 hour. Destain in Coomassie Blue G-250 Destaining Solution for 3 x 15 minutes washes or until the desired destain is reached.

**Note:** Peptides are not completely fixed and may diffuse out of the gels if fixing and staining times are greatly exceeded. We recommend a maximum fix time of 45 minutes and a maximum staining time of 1.5 hours.

### Polypeptide Fixative Solution

|                 |        |
|-----------------|--------|
| Methanol        | 400 ml |
| Acetic acid     | 100 ml |
| Deionized water | 500 ml |

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**Fig. 1.** SDS polyacrylamide gels run in the Mini-PROTEAN® II cell according to the method of Schagger and von Jagow. Polypeptide SDS-PAGE standards run on a 16.5% Tris-Tricine gel, stained with Coomassie G-250.