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ProductInformation

ColorBurst™ Electrophoresis Marker
(M.W. 8,000–220,000)

Catalog Number **C1992**
Storage Temperature –20 °C

TECHNICAL BULLETIN

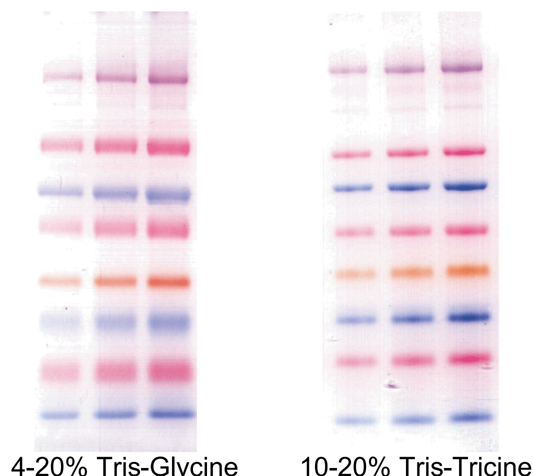
Product Description
ColorBurst™ Electrophoresis Markers are designed for qualitative molecular mass determinations in Laemmli SDS-PAGE systems,[1] and for visual confirmation of Western blot transfer efficiency.

ColorBurst Markers are ready-to-use. They are formulated in a solution that resists freezing.

ColorBurst Markers offer the following features and benefits:

- No need for chemical reduction of the markers before loading the gel.
- No boiling required.
- No freeze/thaw cycles confers diminished degradation and longer shelf life.
- Storage at –20 °C saves on precious –70 °C freezer space.
- Simply remove from the freezer, warm to room temperature, and load the gel.

Figure 1.
ColorBurst Marker in SDS-PAGE Gradient Gels



4-20% Tris-Glycine   10-20% Tris-Tricine

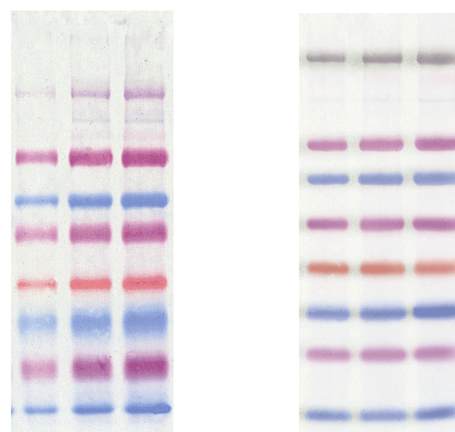
Both gels were loaded (left to right) with 3, 5 and 7 µl of the **ColorBurst** Marker. The markers were run using standard conditions on 10 × 10 cm, 1 mm thick, 10-well precast gels.

ColorBurst Markers are composed of 8 proteins, which have been chemically reduced, alkylated, and conjugated to brilliantly colored dyes. They can be readily visualized on a gel or membrane after transfer. Each vial of **ColorBurst** Markers contains 500 µl of solution, enough for at least 50 mini-gel applications.

ColorBurst Markers transfer cleanly to nitrocellulose or PVDF membranes using Towbin's[2] or CAPS buffers, respectively.

Storage/Stability
This product ships on wet ice and storage at –20 °C is recommended. **ColorBurst** Markers are stable for at least one year as supplied. Crystals may form in the solution during storage at –20 °C. These crystals dissolve readily upon warming to room temperature. Repeated crystal formation will not affect the performance of the **ColorBurst** Markers.

Figure 2.
ColorBurst Marker transferred to nitrocellulose membranes using Towbin's buffer.[2]



Bands transferred to nitrocellulose membranes from the gels in Figure 1 (Tris-Glycine on left and Tris-Tricine on right). Transfers were completed in 90 minutes at 70 volts with Towbin's buffer (Tris-Glycine in 20% methanol.)

Apparent Molecular Masses (kDa) of ColorBurst Marker Proteins		
Band Color	4–20% Gel Tris-Glycine	10–20% Gel Tris-Tricine
Violet	220	210
Pink	100	90
Blue	60	65
Pink	45	40
Orange	30	30
Blue	20	20
Pink	12	13
Blue	8	8

Apparent molecular masses were determined using SigmaMarker, Wide Range (6.5–200 kDa) as a comparison standard. The molecular mass of the violet band, which is outside the range of the standard, is an approximation.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Note: It is **not** recommended that ColorBurst Markers be used as standards for quantitative molecular mass determinations, but only as a qualitative tool. For quantitative molecular mass determinations, use the appropriate SigmaMarker product, which can be found at www.sigmaaldrich.com.

Procedure

ColorBurst Markers are supplied ready-to-use. Remove from the freezer and warm to room temperature before loading onto the gel.

The following are suggested loading volumes for various gel formats:

- 1–2 μl for a PhastGel®
- 5–10 μl for a mini-gel with no transfer
- 3–5 μl for a mini-gel with transfer to a membrane
- 10–15 μl for a large gel

Note for Chemichrome (C2117 and C2242) Users

Additional protein controls may be loaded into the same lane as the ColorBurst marker to provide a positive control when performing a Western blot. Two examples would the addition of the primary detection antibody or the addition of the target protein. In some cases, both can be added. A general guide is presented, while the exact experimental parameters are to be finalized by the researcher.

A. Addition of primary antibody
   1. Prepare the primary antibody in Laemmli 2× sample buffer (Catalog Number S3401) at a concentration of 0.05–0.5 mg/ml and heat at 95–100 °C for 5 minutes to completely denature the protein.
   2. Add up to 5 μl of this control sample to the same well as the ColorBurst Marker.
      Notes: Suitable controls for common IgG antibodies are as follows: mouse IgG (Catalog Number I8765), rabbit IgG (Catalog Number I5006), and goat IgG (Catalog Number I5256).

      The specific primary antibody used for detection can also be used.

B. Addition of target protein – verification that either the primary antibody or secondary antibody is working properly in the Western blot analysis.
   1. Prepare the purified target protein or crude protein extracts known to contain the target protein in Laemmli 2× sample buffer (Catalog Number S3401) at a concentration of 0.05–0.5 mg/ml and heat at 95–100 °C for 5 minutes to completely denature the protein.
   2. Add up to 5 μl of this control sample to the same well as the ColorBurst Marker.
      Notes: The optimal level of target protein must be determined by the user. The amount of target protein to use will depend upon the antibodies used and the detection method used in the Western blot.

      As a general rule, more purified target protein samples will require less total protein compared to crude samples.

References

1. Laemmli, U.K., Nature, **227**, 680 (1970).
2. Towbin, H. *et al.*, Proc. Natl. Acad. Sci. USA, **76**, 4350 (1979).

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PhastGel is a registered trademark of GE Healthcare.

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