

BenchMark[™] Fluorescent Protein Standard

Cat. no. LC5928	Size 125 μL	Store at -30°C to -10°C
Pub. Part no. LC5928.pps	MAN0001524	Rev. Date: 17 January 2012

Description

The BenchMark[™] Fluorescent Protein Standard allows easy and direct visualization of molecular weight ranges of proteins labeled with Lumio[™] Green Detection Kit (Cat. no. LC6090) or other fluorescent-conjugated proteins after SDS-PAGE. The BenchMark[™] Fluorescent Protein Standard consists a set of wide molecular weight range proteins conjugated to a fluorescent dye, Alexa Fluor[®] 488.

The important features of the standard are listed below:

- Consists of 7 distinct protein bands in the range of ~11-155 kDa
- Fluorescence spectra* of the dye is compatible with excitation and emission wavelengths of common instruments allowing direct visualization of standard protein bands on a UV transilluminator or a visible light laser-based scanning instrument after SDS-PAGE
- Visualized also with Coomassie stains on SDS-PAGE gels
- Suitable for NuPAGE® Novex Gels or Tris-Glycine Gels

*The maximum excitation of the dye is at 493 nm and maximum emission is at 516 nm, see www.lifetechnologies.com for fluorescence spectra.

Specifications

Contents:	125 μL of BenchMark [™] Fluorescent Protein Standard
Storage	0.45 M Tris-HCl, pH 8.5; 2% SDS; 12% glycerol;
Buffer:	0.0025% Coomassie G-250
Storage:	Store at -30° C to -10° C. To avoid freezing and thawing, aliquot in small volumes and store at -30° C to -10° C in dark.
Stability:	6 months at -20°C

Product Use For research use only. Not for human or animal therapeutic or diagnostic use.

Directions

The BenchMark[™] Fluorescent Protein Standard is supplied in a ready-to-use format. There is no need to heat or add reducing agent.

- 1. Thaw the ladder at room temperature. Vortex gently to ensure the solution is homogenous.
- 2. Load the ladder into the well of a SDS gel using the loading volumes listed below to obtain the best results:

Gel	Electrophoresis/Blotting	
Mini-Gel	2–5 μL	
Standard Gel	5–10 µL	

3. After electrophoresis, place the gel on a UV transilluminator equipped with a standard camera and select the ethidium bromide filter to visualize standard protein bands as shown on the next page. You can also use a visible light laser-based scanner with appropriate filters (see page 1 for excitation and emission maxima of the dye).

Note: For fluorescence spectra of the dye and more details on visualizing the gel, visit **www.lifetechnologies.com**.

4. Image the gel with a standard, CCD, or video camera with appropriate filters. You may need to expose the gel for different time periods to obtain the best image. The dye has high photostability allowing longer exposure times for maximum sensitivity.

Note: The standard can also be stained with Coomassie stain using your method of choice.

Product Qualification

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available at **www.lifetechnologies.com/support**, and is searchable by product lot number, which is printed on each box.

Blotting

After transferring proteins to a suitable membrane, visualize the standard protein bands on the membrane using any method described below.

- Place the **wet** membrane on a UV transilluminator equipped with a standard camera. Visualize and image the membrane by exposing the membrane to UV light from the bottom.
- Place the dried membrane on a UV transilluminator equipped with a standard camera. Visualize and image the membrane by exposing the membrane to UV light from the top (you may place the UV transilluminator on its side to illuminate the blot or use a top illuminating system).
- Use a visible light laser-based scanner with appropriate filters to visualize and image the membrane by epi-illumination (reflective).

Example

The apparent molecular weights of the protein bands in BenchMark[™] Fluorescent Protein Standard in different gel buffer systems are shown below. BenchMark[™] Fluorescent Protein Standard (5 µL) was separated on a NuPAGE[®] Novex 4–12% Bis-Tris Gel with MES buffer (see figure below) and visualized on a UV transilluminator using a 3–4 second exposure.

_	Band No.	Approximate Molecular Weights (kDa)		
1		NuPAGE® 4-12%	4–20%	
2		Bis-Tris Gel with	Tris-Glycine	
з —		MOPS/MES	Gel	
	1	155	155	
5	2	98	100	
Ĩ	3	63	65	
6 —	4	40	41	
	5	32	33	
7	6	21	23	
	7	11	12	

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