

68721 LUCY® 506 Solution

Application

LUCY® 506 is a fluorescent stain for protein gelelectrophoresis, with high sensitivity and easy, fast and robust staining procedure for all kinds of SDS gels. Protein staining by LUCY® 506 does not interfere with subsequent MALDI-MS. In addition, LUCY® 506 can also be used for protein quantification in solution.

Product Description

Spectral data: $\lambda_{ex}=506 \text{ nm}$ / $\lambda_{em}=520 \text{ nm}$
 Contents: LUCY® 506 is provided as a 5000 x stock-solution in DMF (5 mg/ml)
 Sensitivity: LOD: 3-10 ng/band
 Linearity: Linear between 3 and 1000 ng/band
 Handling: Do not expose to light unnecessarily
 Reuse: Reuse of the dye will result in reduced sensitivity

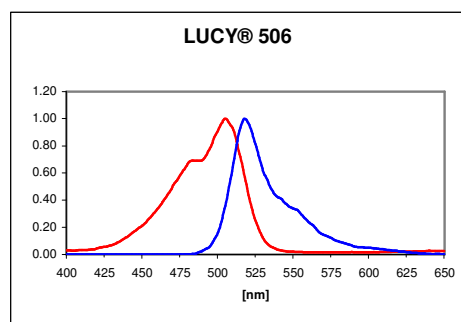


Fig.1 Normalized fluorescence excitation (red) and emission (blue) spectra of LUCY® 506 ($5 \cdot 10^{-5} \text{ M}$) in the presence of BSA (0.2 mg/ml) and SDS (0.05 %)

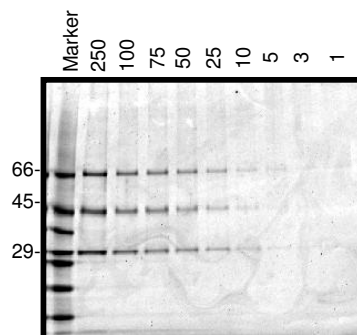


Fig.2 10-20 % Tris-Glycine gel, run in 0.05 % SDS. 3 proteins/lane (1-250 ng/band), stained with LUCY® 506, imaged on FLA-3000.

Staining procedures

Staining of Mini-Gels, standard protocol (1D or second dimension of 2D; 1mm thickness):

- 1) Electrophoresis is performed under standard conditions, using 0.1 % SDS in the running-buffer (or 0.05 % SDS for reduced background-staining)
- 2) The gel is immersed in 50 ml 1 x staining solution (10 μl Lucy-506 in 50 ml 7.5 % acetic acid) for 60 min in the dark on a rocking table. Higher dye-concentrations will result in increased background staining
- 3) Rinse the gel with 7.5 % acetic acid for 30 s
- 4) Short water rinse before imaging

Prestaining method by adding dye to the cathode-buffer:

20 μl LUCY® 506 (5 mg/ml stock in DMF) is added to 120 ml of 1x running-buffer (10 x buffer = 250 mM Tris / 1.92 M Glycine / 0.5 % SDS / pH 8.3) in the cathode-buffer-compartment. The same buffer is used for the anode-compartment, but without dye. After the run, destaining in 7.5 % acetic acid for 15-60 min is necessary to remove unspecific background.

It is not possible to pre-stain the samples themselves, before loading them onto the gel.

Staining of gels with a plastic backing:

Phast-gels or Dalt 12.5 gels may be stained using the standard protocol, however with reduced sensitivity due to autofluorescence of the backing

Staining of large 2D-Gels:

- 1) The 2D-gel is stained for 2 h in the dark (80 µl LUCY® 506 in 400 ml 7.5 % acetic acid)
- 2) Destain for 30 s in 7.5 % acetic acid
- 3) Short water rinse before imaging

Native Gels:

- 1) Run the electrophoresis in SDS-free buffers
- 2) Rinse the gel for 30 min in 0.05 % SDS
- 3) Stain the gel for 60 min in the dark in 50 ml 1 x staining solution
- 4) Rinse the gel with 7.5 % acetic acid for 30 s
- 5) Short water rinse before imaging

Detection

Detection is performed by illuminating the gel on a blue light transilluminator (Dark-Reader, Clare Chemical Research), and imaging the gel using a Gel-Logic-100 (Kodak, 1-3 s, f-stop 3-5) with a 590 nm band-pass filter. Alternatively, a UV screen may be used for excitation and a CCD-camera with a 535 nm band-pass filter for detection. Moreover, detection may be performed on a laser-scanner (FLA-3000, Fuji), using 473 nm excitation and 520 nm emission-filter, or by using a Polaroid Camera.

Other imaging systems are possible with the corresponding excitation sources and emission filter settings. Try to minimize the exposure to light, work quickly!

Problems / interfering substances

Do not use organic solvents during destaining or fixing (MeOH, EtOH), as it will strip off dye and SDS

Tested gel-systems

- Tris-Glycine (Laemmli)
- Nupage Bis-Tris (with MOPS-buffer)
- Dalt Gel 12.5 (GE)
- PhastGel (GE)

Storage

Protect from light; store at 4 °C

Note

Overall three new fluorescent protein gel stains, LUCY® 506, LUCY® 565 and LUCY® 569 are available. The profile of each of the dyes is different: LUCY® 506 shows highest sensitivity on SDS gels, LUCY® 565 allows neutral staining (ideal e.g. for subsequent Western blotting) and LUCY® 569 excels by an extraordinary broad linear dynamic range.

Special Application: Protein Quantification in Solution

LUCY® 506 can be used to quantify proteins in solution.

This application can be performed in cuvettes (read-out in a fluorescence spectrometer), or in a 96-well microplate with glass-bottom (read-out on Laser-Scanner or fluorescence microplate reader).

It is applicable for 2 different protein concentration ranges.

1. Low range (linear range < 50 µg/ml Protein):

2. High range (linear range 10-1000 µg/ml Protein):

The following solutions are required:

1. 50 mM Phosphate buffer (pH 5.0)
2. 0.1 % SDS solution
3. Lucy-506 (diluted to 0.1 mg/ml in DMF)

The following solutions are required:

1. 50 mM Tris buffer (pH 8.0)
2. 0.05 % SDS solution
3. Lucy-506 (diluted to 0.1 mg/ml in DMF)

- Use known concentrations of BSA as a standard
- Dilute the BSA standard and the sample protein respectively in the buffer portion
- Mix the the solutions according to this table and measure immediately:

	well [µl]	cuvette [µl]
Buffer (containing BSA / sample protein)	100	1000
SDS	100	1000
LUCY® 506	5	50

- Compare the measured fluorescence values of the unknown sample with the BSA values and calculate the concentration of the sample on that basis

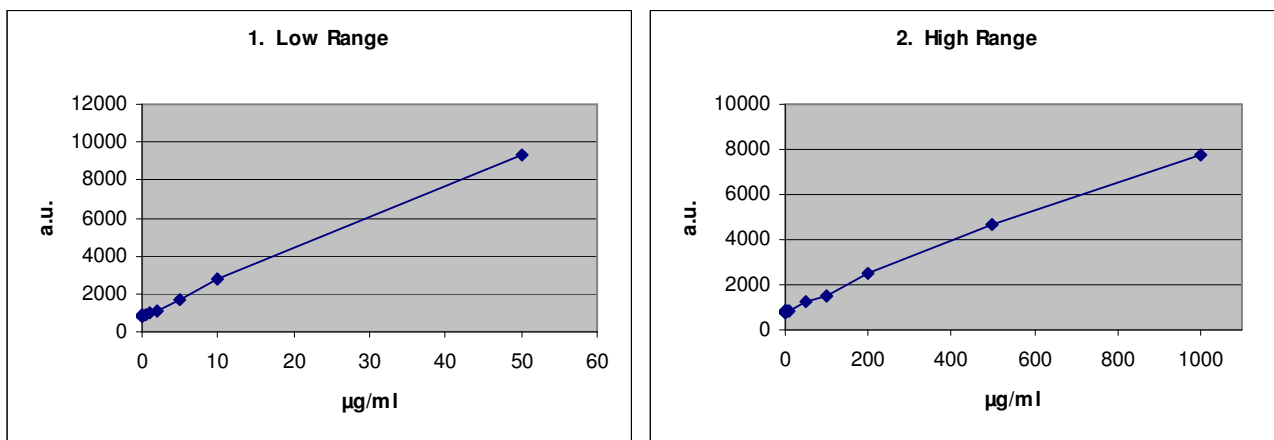


Fig.3 Different concentrations of BSA in solution were quantified in the 96-well microplate format using LUCY® 506. Low range (left) and high range (right). Detection was done on Fuji FLA-3000 ($\lambda_{ex}=473$ nm / $\lambda_{em}=520$ nm).

Interfering substances:

Triton X-100, Tween-20, EDTA, Urea, NaCl, organic solvents