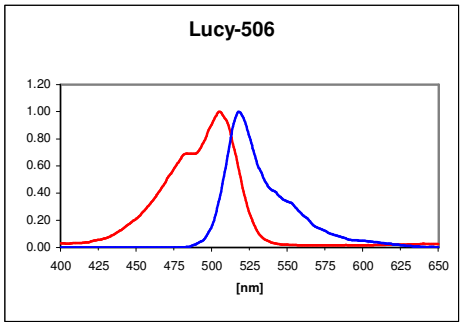
14149 Lucy-506 Protein Gel Stain

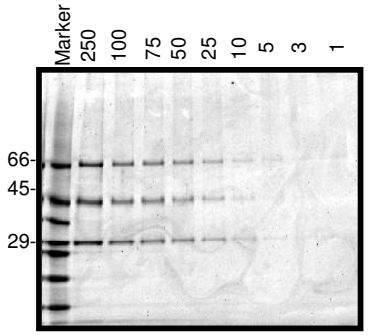
Application
Lucy-506 is a fluorescent stain for protein electrophoresis, with high sensitivity and easy, fast and robust staining procedure for all kinds of SDS gels. Protein staining by Lucy-506 does not interfere with subsequent MALDI-MS.

Product Description

Spectral data:	λex=506 nm ; λem=520 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:	Warm to room temperature before opening. Do not expose to light unnecessarily.
Reuse:	Reuse of the dye will result in reduced sensitivity



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



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 (inverted).

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) are 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 prestain the samples themselves, before loading them onto the gel.

Industriestrasse 25
CH-9471 Buchs
E-mail: Flukatec@europe.sial.com
Fax: ++41 81 755 2815

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 sec., f-stop 3-5) with a 590 nm band-pass filter or a laser-scanner (FLA-3000, Fuji), using 473 nm excitation and 520 nm emission-filter or a Polaroid Camera. Try to minimize the exposure to light, work quick!

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 12,5 (Amersham)
- PhastGel (Amersham)

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 (e.g. for subsequent Western blotting) and Lucy-569 excels by an extraordinary broad linear dynamic range.

Industriestrasse 25
CH-9471 Buchs
E-mail: Flukatec@europe.sial.com
Fax: ++41 81 755 2815