

43772 LUCY[®] 565 Solution

Application

LUCY[®] 565 is a fluorescent stain for protein gelelectrophoresis, with high sensitivity and easy, fast and robust staining procedure for all kinds of SDS gels. LUCY[®] 565 is also suitable for neutral staining (e.g. for subsequent western blotting).

Protein staining by LUCY[®] 565 does not interfere with subsequent MALDI-MS.

Product Description

Spectral data: $\lambda_{ex}=565 \text{ nm}$ / $\lambda_{em}=588 \text{ nm}$

Contents: LUCY[®] 565 is provided as a 5000 x stock-solution in DMSO (5 mg/ml)

Sensitivity: LOD: 5-10 ng/band

Linearity: Linear between 5 and 4000 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

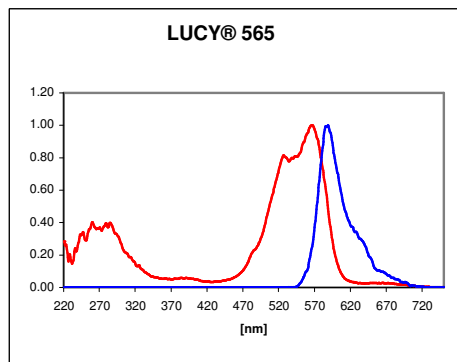


Fig.1 Normalized fluorescence excitation (red) and emission (blue) spectra of LUCY[®] 565 ($5 \cdot 10^{-5} \text{ M}$) in the presence of BSA (0.2 mg/ml) and SDS (0.05 %)

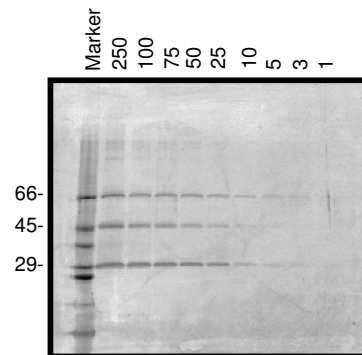


Fig.2 4-20 % Tris-Glycine gel, run in 0.05 % SDS. 3 proteins/lane (1-250 ng/band), stained with LUCY[®] 565, imaged on FLA-3000.

Staining procedures

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

- 1) Electrophoresis is performed under standard conditions, using 0.05 % SDS in the running-buffer for reduced background-staining
- 2) The gel is immersed in 50 ml 1 x staining solution (10 μl LUCY[®] 565 in 50 ml water) for 60 min in the dark on a rocking table. Higher dye-concentrations will result in increased background staining
- 3) Rinse the gel with water for 30 s
- 4) Imaging

Alternative staining protocol, including a fixation step:

- 1) The gel can be fixed in 5 % trichloroacetic acid after the run for 30 min, and then soaked in a 0.05 % SDS solution for 30 min
- 2) The gel is stained in 50 ml NaOAc solution (1 M, pH 4.5) for 45 min, containing 10 μl LUCY[®] 565
- 3) 30 s destain in 1 M NaOAc
- 4) short water rinse before imaging

Prestaining method by adding dye to the cathode-buffer:

20 µl LUCY[®] 565 (5 mg/ml stock in DMSO) are added to 120 ml of 1 x 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:

If a Westernblot is planned afterwards, perform the stain and destain in water instead of acetic acid.

- 1) The 2D-gel is stained for 2 h in the dark (80 µl LUCY[®] 565 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 sec
- 5) Short water rinse before imaging

Gel stains + Western Blotting:

It is possible to stain a gel and perform a Westernblot afterwards:

- 1) Stain the gel with 10 µl Lucy-565 in 50 ml water (not in acetic acid and without fixation before!)
- 2) The gel is immersed for 60 min in the dark on a rocking table
- 3) Short water rinse before imaging
- 4) Continue with Westernblot-Transfer

It is not possible to use LUCY[®] 565 for staining proteins on westernblot membranes

Detection

Detection is performed by illuminating the gel on a UV-screen, and imaging the gel using a Gel-Logic-100 (Kodak, 1-3 s, f-stop 3-5) with a 590 nm band-pass filter or a laser-scanner (FLA-3000, Fuji), using 532 nm excitation and 580nm emission-filter or 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 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.