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Product Information

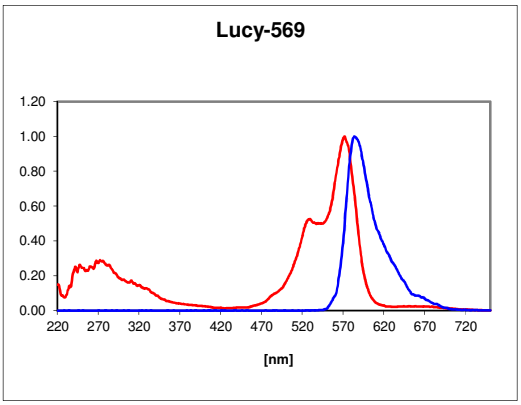
41629 Lucy-569 Solution

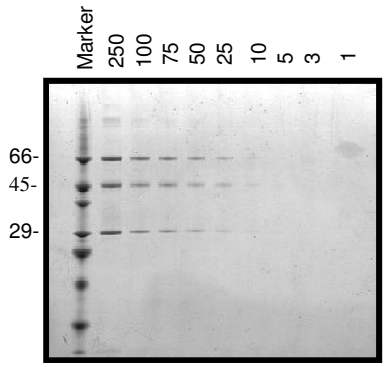
Application

Lucy-569 is a fluorescent stain for protein gelelectrophoresis, with good sensitivity and easy, fast and robust staining procedure for all kinds of SDS gels.
Lucy-569 provides a broad linear dynamic range for protein quantification in electrophoresis (5-6000 ng protein per band). Protein staining by Lucy-569 does not interfere with subsequent MALDI-MS.
In addition, Lucy-569 can also be used for protein quantification in solution.

Product Description

Spectral data:	λex=569 nm / λem=585 nm
Contents:	Lucy-569 is provided as a 5000 x stock-solution in DMSO (5 mg/ml)
Sensitivity:	LOD: 5-10 ng/band
Linearity:	Linear between 5 and 6000 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





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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 1x staining solution (10 µl Lucy-569 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

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 -569
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-569 (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 prestain 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-569 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 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 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-569 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):

The following solutions are required:

1. 50 mM Phosphate buffer (pH 5.0)
2. 0.1 % SDS solution
3. Lucy-569 (diluted to 0.1 mg/ml in DMSO)

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

The following solutions are required:

1. 50 mM Tris buffer (pH 8.0)
2. 0.05 % SDS solution
3. Lucy-569 (diluted to 0.1 mg/ml in DMSO)

- 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-569	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

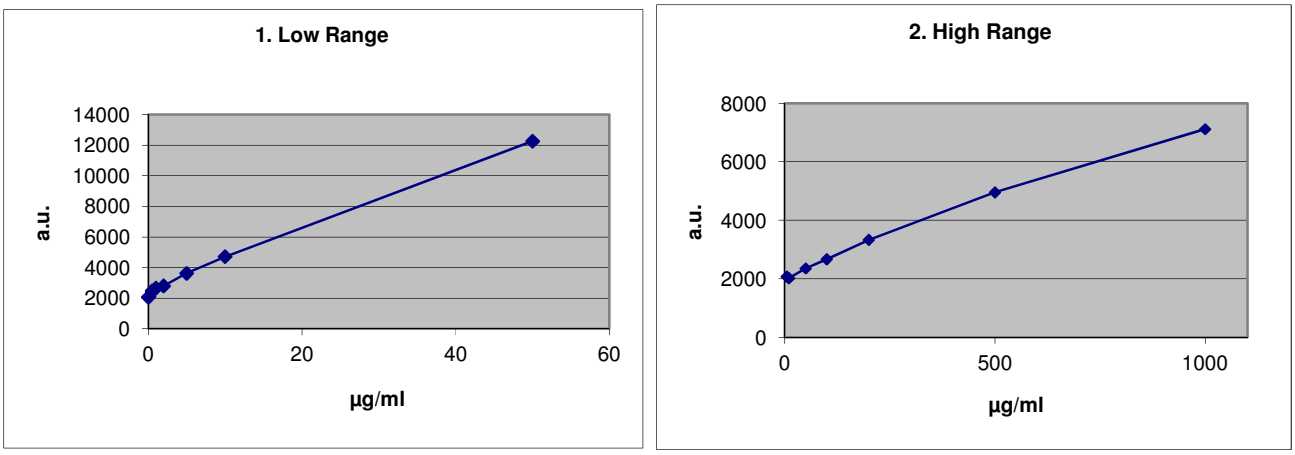

Fig.3 Different concentrations of BSA in solution were quantified in the 96-well microplate format using Lucy-569. Low range (left) and high range (right). Detection was done on Fuji FLA-3000 (λex=532 nm / λem=580 nm).

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

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.