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: $\lambda_{\text{ex}}=506 \text{ nm} / \lambda_{\text{em}}=520 \text{ nm}$

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

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 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-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):
   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)

   • 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:

<table>
<thead>
<tr>
<th></th>
<th>well [µl]</th>
<th>cuvette [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (containing BSA / sample protein)</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>SDS</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>Lucy-506</td>
<td>5</td>
<td>50</td>
</tr>
</tbody>
</table>

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

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-506 (diluted to 0.1 mg/ml in DMF)

   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.

©2016 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.

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 (λex=473 nm / λem=520 nm).