SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

51723 LUCY® Molecular Weight Standard Kit

Kit components

- 43772 LUCY 565 solution (2 vials)
- S8445 SigmaMarker™, Wide Range (1 vial)
- Technical information sheet

Description

The “LUCY 565 Molecular Weight Standard Kit” contains the fluorescent protein stain LUCY 565 and a Molecular Weight Marker, consisting of 12 proteins. The MW-Marker allows the precise size determination of proteins on electrophoresis gels, and LUCY 565 allows a highly sensitive, easy, fast and robust staining procedure on various kinds of SDS gels.
LUCY 565 is suitable for neutral staining, i.e. a Western blot can be performed from a gel that has previously been stained with LUCY 565.
Protein staining by LUCY 565 does not interfere with subsequent MALDI-MS.

Storage

Protect from light; store kit at 4 °C; freezing is not recommended.

Molecular Weight Marker

The Sigma MW-Marker contains 12 proteins in the range of 6.5 – 200 kDa.
The vial contains the protein mixture in lyophilized form.

Preparation of the marker:

1. Add 100 µl water and reconstitute the marker by vortexing
2. Do not heat!
3. Immediately aliquot and store the unused portions at -20 °C. Repeated freezing and thawing of reconstituted SigmaMarker is not recommended.

LUCY 565

Spectral data:	λex=565 nm / λem=588 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 and is not recommended

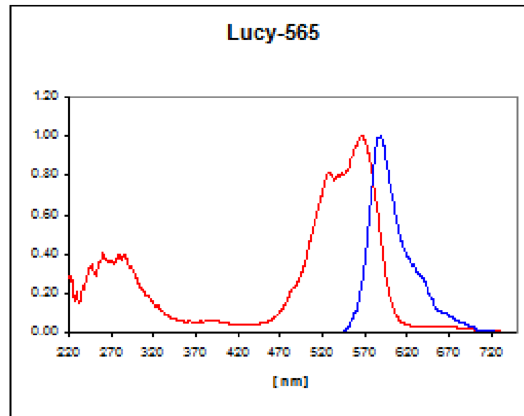

Fig.1 Normalized fluorescence excitation (red) and emission (blue) spectra of LUCY 565 in the presence of BSA (0.2 mg/ml) and SDS (0.05 %)

Standard protocol (staining of 1D Mini-Gels, or second dimension of 2D; 1 mm thickness)

1. Add 5 µl MW-Marker to a mini-gel (recommended dilution 1:5, see Fig.2)
2. Electrophoresis is performed under standard conditions (0.05 or 0.1 % SDS in the running-buffer)
3. After the run, 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
4. Rinse the gel with water for 30 s
5. Imaging

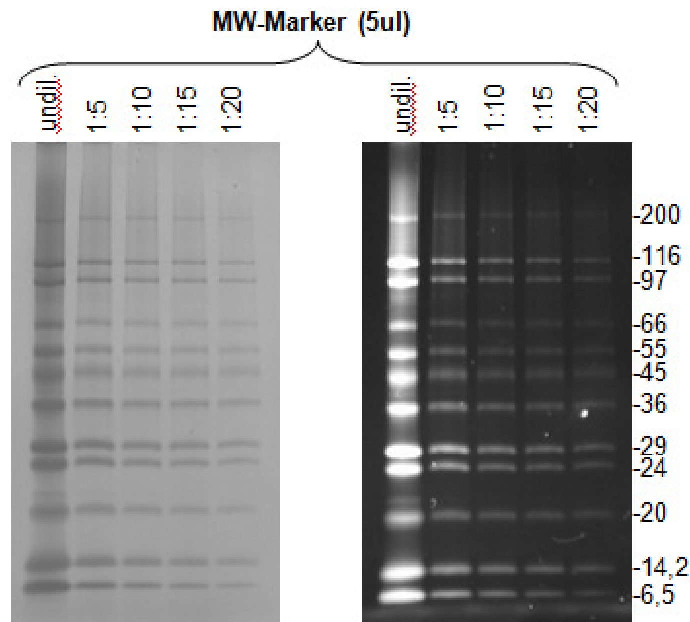

Fig.2 4-20 % Tris-Glycine gel. 5 µl MW-Marker per lane in different dilutions, stained with LUCY 565. Left: imaged on FLA-3000 (ex 532 nm / em 580 nm), right: UV-screen, CCD-camera (Gel Logic 100), 590 nm filter.

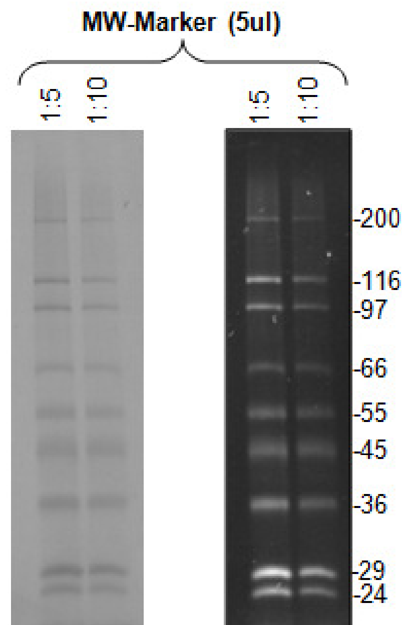

Fig.3 10 % Tris-Glycine gel. 5 µl MW-Marker per lane in different dilutions, stained with LUCY 565. Left: imaged on FLA-3000 (ex 532 nm / em 580 nm), right: UV-screen, CCD-camera (Gel Logic 100), 590 nm filter.

Additional staining protocols

Gel stains + Western Blotting:
It is possible to stain a gel and perform a Western blot 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 Western blot transfer

It is not possible to use LUCY 565 for staining proteins on Western blot membranes.

Staining of large 2D-Gels:

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

Staining of gels with a plastic backing:
Phast-gels or Dalt 12.5 gels may be stained using the standard protocol, however they show reduced sensitivity due to autofluorescence of the backing.

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

Related products

Description	Cat. No.	Package size
LUCY 506	68721	500 µl
LUCY 565	43772	500 µl
LUCY 569	41629	500 µl
LUCY Starter Kit	51153	1 Kit
Laemmli Lysis-buffer, non-smelling	38733	5 x 2 ml

Nupage® is a registered trademark of Invitrogen.
Ettan™ Dalt and PhastGel™ are registered trademarks of GE Healthcare.

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.