New Fluorescence Dyes For Protein Gel Stains

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Summary

Three new fluorescent protein gel stains, LUCY-506, LUCY-565 and LUCY-569 have been developed. The profile of each of the dyes has been established: LUCY-506 shows highest sensitivity. LUCY-565 allows neutral gel-staining (e.g. before Western blotting) and LUCY-569 excels by a linear response over an extraordinary broad linear dynamic range.

Motives

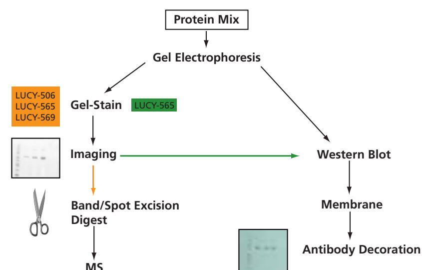
- Standard procedures for on gel protein staining either lack sensitivity for the lower ng/band protein range (e.g. Coomassie Blue stains), are labour-intensive (silver stains), or show poor MS-compatibility.
- Commercially available fluorescent stains overcome those disadvantages only partially and are generally expensive.
- Conventional stains are not designed for state-of-the art detectors such as CCD camera imagers and laser scanners and generally show limited linearity in signal intensity vs. protein/band relation which make them unsuitable for protein quantification on gel.

Staining Protocols

	Standard procedure	Prestaining procedure	Stain after fixation	Native PAGE stain	Neutral stain before WB
	post-electrophoretic stain in HOAc; time required: 1h	dye in cathode-running-buffer with subsequent destaining in HOAc; time required: 15-60 min	TCA-fixation; SDS-rinse; stain in NaOAc; time required: 1h 45 min	run gel SDS-free; rinse gel in SDS; then standard procedure; time required: 1h 30min	postelectrophoretic stain in water; time required: 1h; continue with Western-Blot transfer
LUCY-506	+++	++	+	+++	-
LUCY-565	+	+	++	+++	+++
LUCY-569	+++	++	++	+++	-

Table 1. Suitability and performance of LUCY stains for various staining procedures.

Detection Workflow



Optimum Detection Settings

Imaging Device	Illumination	LUCY-506 Filter	LUCY-506 Sensitivity	LUCY-565 Filter	LUCY-565 Sensitivity	LUCY-569 Filter	LUCY-569 Sensitivity
Polaroid Camera	Dark Reader® ($\lambda_{max.}$ ~450 nm)	orange filter	+		not tested		not tested
CCD Camera	UV screen ($\lambda_{max.}$ ~310 nm)	535 nm band pass	++		not tested		not tested
		590 nm band pass	-	590 nm band pass	++	590 nm band pass	+++
	Dark Reader® ($\lambda_{max.}$ ~450 nm)	590 nm band pass	+++	590 nm band pass	+	590 nm band pass	+
		amber filter	+	amber filter	-	amber filter	-
Laser Scanner	473 nm laser	520 nm cut off	+++	520 nm cut off	-	520 nm cut off	-
	532 nm laser	580 nm cut off	-	580 nm cut off	+++	580 nm cut off	++

Table 2. Imaging devices, methods and performance.

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Results

Detection Limits

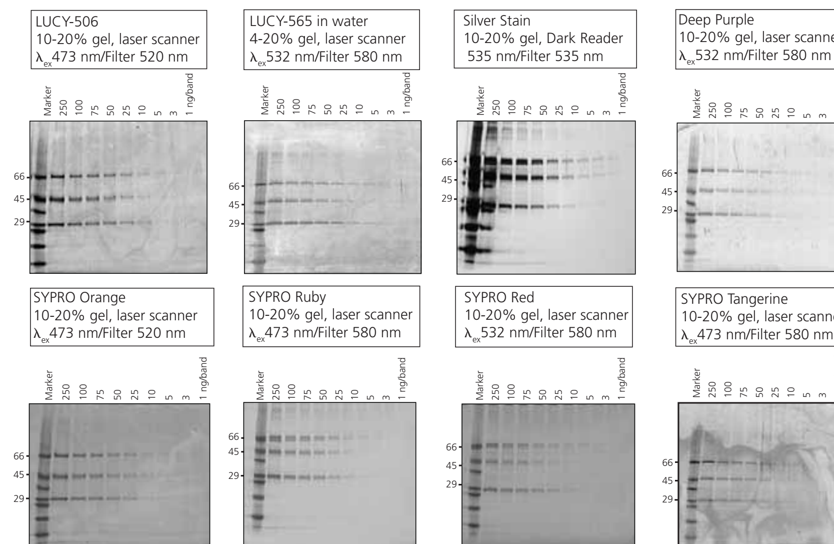


Figure 1. Detection limits of LUCY and SYPRO stains as compared to other common protein stains. A protein standard mixture containing BSA (66 kDa), Ovalbumin(45 kDa) and Carboanhydrase (29 kDa) was used for all gel stains.

2D-Mini-Gels, 10µg *E. Coli*-extract, 7cm IPG-strips pH 3-10, 4-20% Tris-Gly Gel

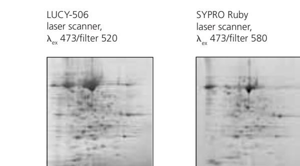
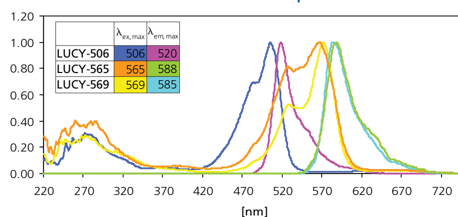


Figure 2. Comparison between LUCY-506 and SYPRO Ruby in 2D-Electrophoresis.

Excitation and Emission spectra



Linear Dynamic Range Determination

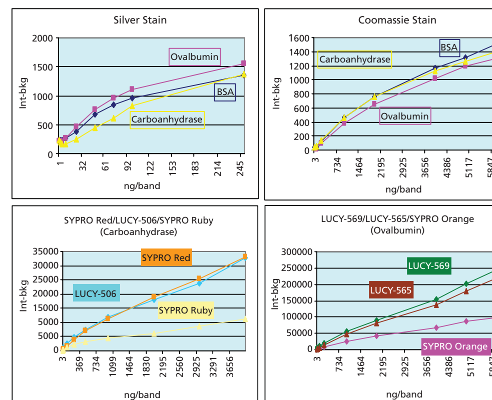


Figure 3. Dynamic range of LUCY stains in comparison to other staining methods.

Mass Spectrometry

Peptide mass fingerprint after in-gel-digest: 100ng E.Coli beta-galactosidase, separated by SDS-PAGE, stained with LUCY 506 (**Spectrum A**) and Sypro Ruby (**Spectrum B**), after band excision and trypsin-digest. Sequence coverage after LUCY-506 stain compared favorably to Sypro Ruby as determined by database analysis.

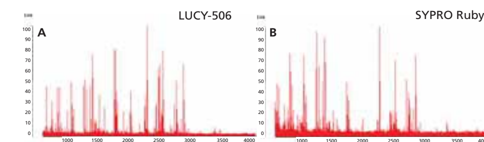
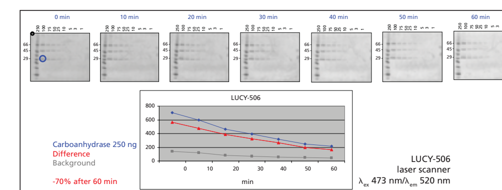


Figure 4. MALDI spectra of extracted peptides, crystallized with HCCA and measured on Shimadzu Kratos CFR MALDI-instrument in reflectron mode.

LUCY-506 Bleaching (60min on UV-screen)



SYPRO Ruby Bleaching (60min on UV-screen)

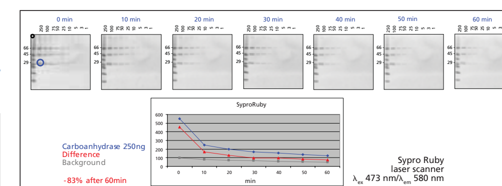


Figure 5. Fluorescence intensity after 60min on a UV-screen. The intensity of the 250ng-Carboanhydrase-band is quantified every 10min (blue: Carboanhydrase; grey: background; red: difference)

Ordering Information

LUCY dyes / LUCY Starter Kit		
Lucy-506 500 µl (5 mg/mL in DMSO) (Cat. No. 14149)	• λ_{ex} 506 nm/λ_{em} 520 nm • sensitivity comparable to SYPRO Orange	• all 3 dyes are compatible with mass-spectrometry • all 3 dyes also stain native PAGEs after SDS-incubation • better sensitivity than Coomassie • better quantifiability than silver • no heavy-metals (SYPRO Ruby, silver) • fast and easy staining protocol (no extra fixation step); 1h total • staining of gels with plastic-backing is possible (with reduced sensitivity) • inexpensive
Lucy-565 500 µl (5 mg/mL in DMSO) (Cat. No. 43772)	• λ_{ex} 565 nm/λ_{em} 588 nm • neutral staining conditions (allows western blot after gel staining)	
Lucy-569 500 µl (5 mg/mL in DMSO) (Cat. No. 41629)	• λ_{ex} 569 nm/λ_{em} 585 nm • broad linear dynamic range	
Lucy Starter Kit 3 x 50 µl (Cat. No. 04297)	• contains all 3 LUCY dyes	

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