

INSTRUCTION MANUAL

SERVA Lightning Sci2

(Cat. No. 43404)

SERVA Lightning Sci3

(Cat. No. 43405)

SERVA Lightning Sci5

(Cat. No. 43406)

SERVA Lightning SciDye Set

(Cat. No. 43407)

Fluorescent dyes for protein detection

SERVA
Electrophoresis

SERVA Electrophoresis GmbH - Carl-Benz-Str. 7 - 69115 Heidelberg

Phone +49-6221-138400, Fax +49-6221-1384010

e-mail: info@serva.de -<http://www.serva.de>

CONTENTS

1. SERVA Lightning SciDyes	2
1.1. General information	2
1.2. Storage conditions	2
2. Labelling of proteins	3
2.1. Workflow of the labelling procedure	3
2.2. Sample preparation	3
2.3. Reconstitution of SERVA Lightning SciDyes	4
2.4. Minimal labelling of lysines	4
2.4.1. Preparation of SciDye stock solution	4
2.4.2. Preparation of the SciDye working solution	5

1. SERVA Lightning SciDyes

1.1. General information

The minimal labelling with SciDyes from SERVA allows high efficient detection and high resolution of minor proteins on 2D gels, especially on film-backed HPE™ gels in 2D Fluorescence Difference Gel Electrophoresis (DIGE). The SciDyes are charge- and size matched. Therefore, identical proteins will show identical migration and separation when run on the same gel.

SERVA Lightning Sci2 is compatible with all Cy2® detection systems. SERVA Lightning Sci3 is compatible with all Cy3®- and SERVA Lightning Sci5 with all Cy5® detection systems. For further details, please see table below.

Cy2®, Cy3®, Cy5®: Trademarks of GE Healthcare Company

Product	Features	Size	Cat. No.
SERVA Lightning Sci2	Fluorescent color: green	5 nmol	43404.01
	Excitation maximum: 490 nm	10 nmol	43404.02
	Emission maximum: 510 nm	25 nmol	43404.03
SERVA Lightning Sci3	Fluorescent color: yellow	5 nmol	43405.01
	Excitation maximum: 555 nm	10 nmol	43405.02
	Emission maximum: 570 nm	25 nmol	43405.03
SERVA Lightning Sci5	Fluorescent color: red	5 nmol	43406.01
	Excitation maximum: 645 nm	10 nmol	43406.02
	Emission maximum: 660 nm	25 nmol	43406.03
SERVA Lightning SciDye Set	Contains a set of Sci2, Sci3 and Sci5	5 nmol	43407.01
		10 nmol	43407.02
		25 nmol	43407.03

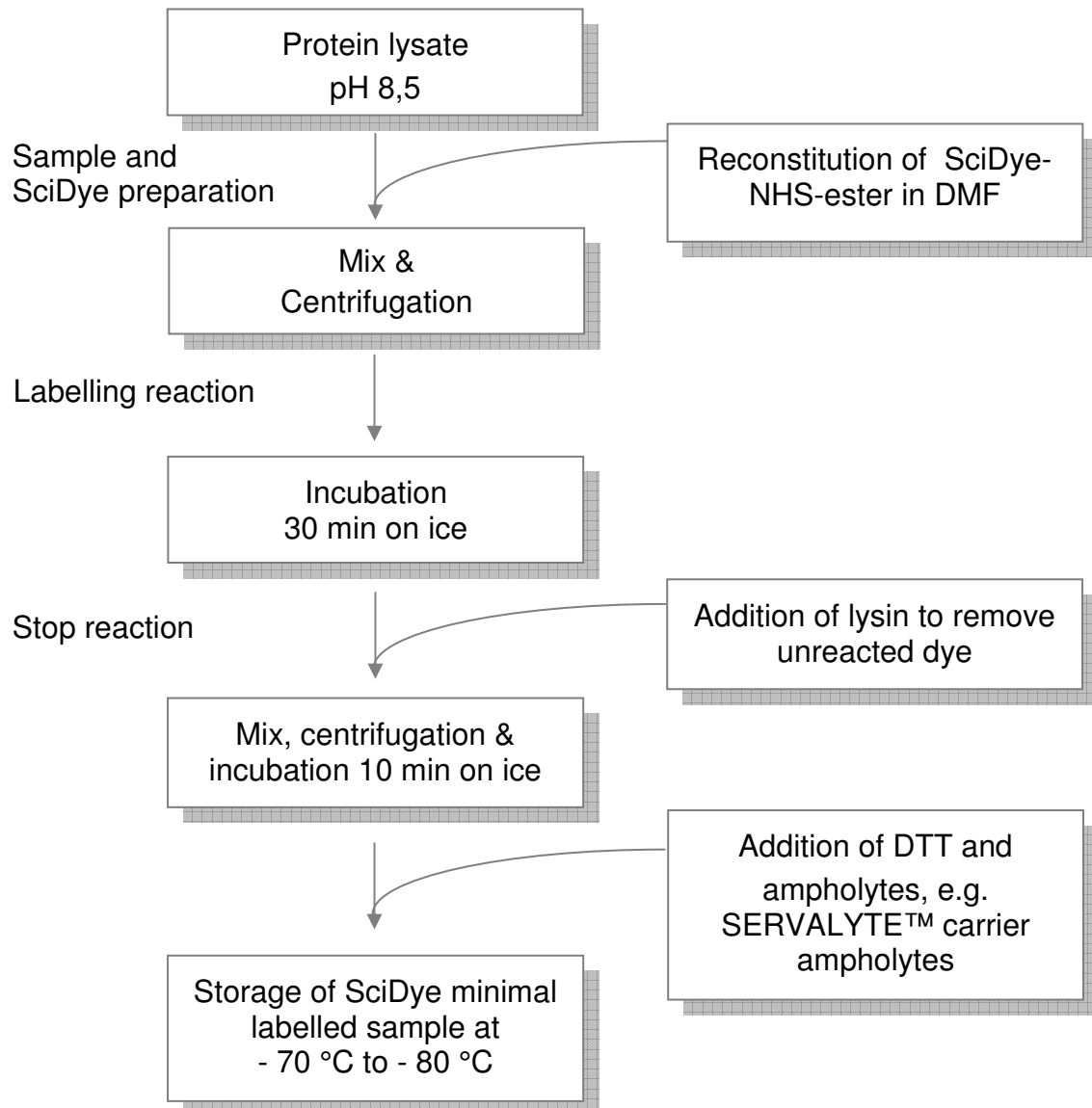
1.2. Storage conditions

If stored at -15 °C to -25 °C, the SERVA Lightning SciDyes will be stable until: see label.

2. Labelling of proteins

SERVA Lightning SciDyes contain a reactive NHS-ester-group to bind covalently at the ϵ -amino group of lysines within the protein sample.

2.1. Workflow of the labelling procedure



2.2. Sample preparation

Please note: During sample preparation always store the samples on ice to minimize protease activity and degradation. To avoid protein sticking on glass surfaces always use plastic lab ware.

Lysis buffer for DIGE:

- Lysis buffer 1: 9 M urea, 4 % (w/v) CHAPS, 30 mM Tris
- Lysis buffer 2: 2 M thiourea, 7 M urea, 4 % (w/v) CHAPS, 30 mM Tris

To adjust pH of the sample: 50 mM NaOH

- Solubilize protein samples either in lysis buffer 1 or in lysis buffer 2.
- Check protein concentration of the samples. Optimally, it would be 5 – 10 mg/ml. The range of 1 – 20 mg/ml is tolerable for successful labelling.
- Check pH carefully. Pipette 2 µl on a pH indicator paper and read out immediately. The pH value should be 8.5. If necessary, adjust with 50 mM NaOH.

2.3. Reconstitution of SERVA Lightning SciDyes

Additionally required: 99.8 % dimethylformamid (DMF), anhydrous

IMPORTANT: The quality of the DMF is critical to ensure successful protein labeling. DMF must be anhydrous and should not be contaminated with water.

After opening, DMF will degrade and form amine compounds that will react the NHS-ester groups of the SciDyes. Addition of 4 Å molecular sieve to DMF during storage will be useful to prolong lifetime.

2.4. Minimal labeling of lysines

2.4.1. Preparation of SciDye stock solution

(Final concentration: 1 nmol/µl)

Solubilize the SciDyes in DMF, anhydrous, to get a final dye concentration of 1 nmol/µl, e.g 25 nmol + 25 µl DMF).

The stock solution show the following colors

- Lightning Sci2: deep yellow
- Lightning Sci3: deep red
- Lightning Sci5: deep blue

- Take a small volume of DMF from it original container and dispense it into a micro centrifuge tube.
- Take SciDye from the – 20 °C freezer and allow to warm 5 min at room temperature.
- Then, add the appropriate volume of DMF to each dye tube (1 nmol/µl = 10³ pmol/µl).
- Close the tubes and mix well (Vortex, 30 s).
- Centrifuge 30 s at 12,000 xg. The SciDye is now ready-to-use.

Please note: If the dyes are not for immediate use, they can be stored light protected at – 20 °C.

2.4.2. Preparation of the SciDye working solution

(Final concentration: 0.4 nmol/ μ l)

- Spin down stock solution (see chapter 2.4.1.).
- SciDye and DMF are mixed 1: 2.5, e.g. 5 μ l SciDye + 7,5 μ l DMF.
- Pipette DMF in a sterile micro centrifuge tube and add SciDye stock solution

IMPORTANT: The SciDye working solution is stable for max. 2 weeks at - 20 °C.

2.4.3. Labelling reaction

Stop solution: 10 mM Lysin (Storage at -20 °C)

IMPORTANT:

- For efficient labeling the sample pH should be 8.5.
- The labeling reaction is performed at 0 °C.
- Optimal protein concentration: 5 – 10 mg/ml
- There should be not carrier ampholytes and reducing agents, e.g. DTT, 2-mercaptoethanol, in the sample.

2.4.3.1. Internal standard: Preparation and Lightning Sci2 labelling

Take the same amount of aliquots from each sample and mix them together. A minimum of 50 μ g protein internal standard per gel is required.

- Pipette a volume of internal standard equivalent to $n \times 50 \mu\text{g}$ (n : number of gels) in a micro centrifuge tube.
- Add $n \mu\text{l}$ Sci2 working solution to the internal standard.

Example for 6 gels ($n=6$):

- Protein amount of the internal standards: $6 \times 50 \mu\text{g} = 300 \mu\text{g}$
- Volume Sci2 working solution: $6 \mu\text{l} = 6 \times 0.4 \text{ nmol} = 2.4 \text{ nmol}$

\Rightarrow 300 μg protein are labelled with 2.4 nmol (= 2,400 pmol) Sci2.

- Mix and centrifuge (12.000 xg, 30 s).
- 30 min incubation on ice (light protected!).
- Add $n \mu\text{l}$ of 10 mM lysine solution, mix and centrifuge (12,000 xg, 30 s).
- 10 min incubation incubation on ice (light protected!).

The labelled standards can now be store at – 70 °C to – 80 °C light protected for min. 3 months.

2.4.3.2. Sample labelling with Lightning Sci3 and Sci5

- Pipette a volume of sample equivalent to 50 µg protein into a micro centrifuge tube.
- Add 1 µl SciDye working solution.

Example:

- Protein amount: 50 µg
- Volume SciDye working solution: 1 µl = 0.4 nmol

⇒ 50 µg protein are labeled with 0.4 nmol (= 400 pmol) SciDye.

- Mix and centrifuge (12.000 xg, 30 s).
- 30 min incubation on ice (light protected!).
- Add n µl of 10 mM lysine solution, mix and centrifuge (12,000 xg, 30 s).
- 10 min incubation incubation on ice (light protected!).

The labelled samples can now be store at – 70 °C to – 80 °C light protected for min. 3 months.