

Biochemicals

**Electrophoresis**

Bioseparation

Life Sciences

Specials

## SERVA Lightning Red for 1D SDS PAGE

Fluorescent Pre-labelling  
of Proteins in 1D SDS PAGE

**The fluorescence dye - Replace gel staining with an easy pre-labelling procedure**

SERVA Lightning Red is a fluorescent dye for rapid labelling of proteins prior to SDS PAGE, making any staining and washing steps after electrophoresis unnecessary. In addition the dye is fully compatible with mass spectrometry and other downstream methods like Western Blotting.

**SERVA Lightning Red has a number of advantages:**

- Direct detection
- No staining and washing steps after the run
- Very high sensitivity, < 0.5 ng BSA
- Wide dynamic and linear range
- No over-staining effects
- Fully MS compatible
- Gel can be further processed by Western blotting

**Unaffected by additives**

SERVA Lightning Red is compatible with all additives typically used for sample solubilisation and protein extraction, including reductants like dithiothreitol (DTT) and dithioerythreitol (DTE).

**No detectable change in electrophoretic mobility**

The shift of the electrophoretic mobility in SDS electrophoresis is very low; migration differences between labelled and non-labelled proteins could not be detected. This is due to the small mass addition of only 288 Da per bound molecule and the low hydrophobicity of the dye.

**SERVA Lightning Red compared to silver and Coomassie® staining**

Traditionally silver staining of PAGE gels is preferred to other staining methods when highest sensitivity of detection is required. However silver stained bands exhibit very quickly saturation, therefore quantification of proteins is not feasible. When gels are compared with the same sample load, silver staining shows bands with higher intensity, however the scan of the fluorescent label displays the bands without limitation in quantification. Compared to SERVA Lightning Red and to silver stained gels Coomassie staining is less sensitive. Easy pre-labelling procedure combined with high sensitivity and excellent quantification properties make SERVA Lightning Red to the fluorescence stain of choice in SDS PAGE gel staining.

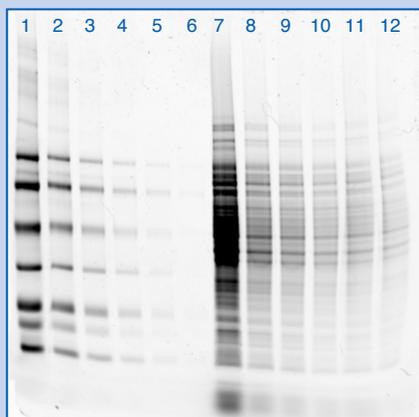


Fig. 1: Stained with SERVA Lightning Red (cat. no. 43401)

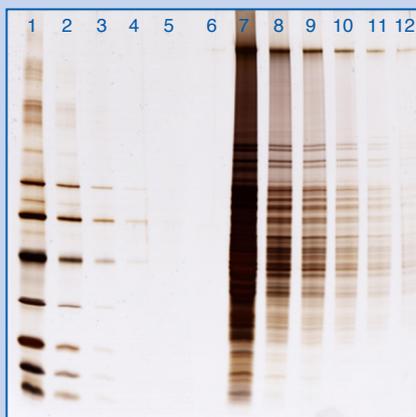


Fig. 2: Stained with SERVA Silver Staining Kit for SDS PAGE (cat. no. 35976)

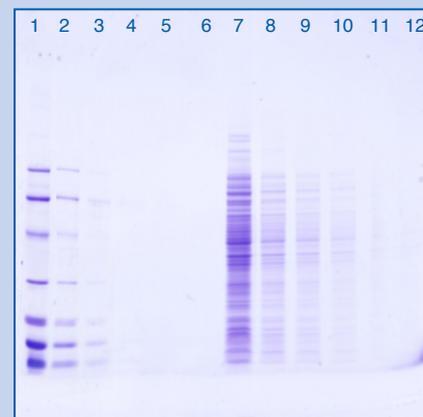


Fig. 3: Stained with Coomassie® Brilliant Blue G (cat. no. 17524)

Fig. 1-3: SERVA<sup>Ge</sup>™ TG PRiME 4–20 %, 12 sample wells, (cat. no. 43276.01). Running conditions: 250V, 50mA, sample volume 5µl serial dilution, total protein content per lane 1-6: Protein Test Mixture for SDS PAGE (cat. no. 39207): 5µg/1µg/0,2µg/40ng/8ng/1,6ng. serial dilution, total protein content per lane 7-12: *E. coli* lysate: 25µg/5µg/2,5µg/1,25µg/0,625µg/0,3125µg

### Easy workflow

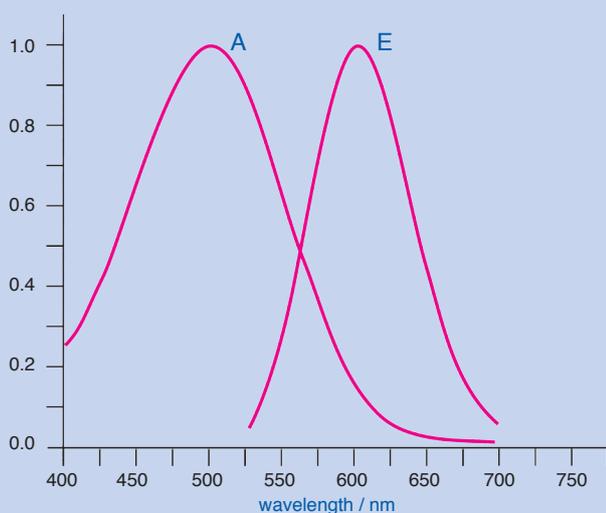
The labelling procedure is simple and quick. When electrophoresis is completed no extra treatment is necessary for detection. Samples of protein concentrations between 1 and 10 mg/ml can be used and no purification or concentration steps after labelling are necessary.

Kits are available for 500 lanes or 1250 lanes – no matter what size of gel - mini, wide or large format - you use.

### How it works

The fluorescence dye binds to primary amino groups, e. g. the  $\epsilon$ -amine of the lysine residues in proteins and peptides. Detection of labelled proteins is performed by fluorescent imager (camera or scanner) at an excitation wavelength of about 530 nm and emission filter of 610 nm with a narrow band width of 30 nm. The bound dye shows a quantum yield (QY\*) of up to 0.60. If bands have to be cut from the gel for mass spectrometry analysis the gels can be fixed in acetic or citric acid and alcohol without any losses in signal intensity for at least 10 days. If required, gels can be post-stained by a preferred method of choice (Coomassie™, silver staining etc.).

\*The fluorescence quantum yield is defined as the ratio of the number of photons emitted to the number of photons absorbed.



A = Absorption spectrum of conjugated form of SERVA Lightning Red  
E = Emission spectrum of conjugated SERVA Lightning Red

### Fluorescent Pre-labelling of Proteins in 1D SDS PAGE

#### Labelling procedure\*

1. Reconstitute the dye in 100  $\mu$ l resp. 250  $\mu$ l water-free DMF (kit dependent), mix well and spin down before making aliquots.
2. Mix the sample with an equal volume of reducing 2x SERVA SDS Sample Buffer pH 8.3. Recommended protein concentration: 1 – 10 mg/ml. Heat the sample for 5 min at 95 °C.
3. Prior to labelling, mix the dye solution 1:50 with 1x reducing sample buffer pH 8.3. Pipet 10  $\mu$ l labelling solution into a vial and add 5  $\mu$ g protein, e. g. 5  $\mu$ l sample with protein concentration of 1 mg/ml or 0.5  $\mu$ l sample with protein concentration of 10 mg/ml. Mix gently and place vial at room temperature for 30 min.
4. Mix gently and apply labelled protein sample on SDS PAGE gel, e. g. 5  $\mu$ l per well.

\*Each kit contains 1 vial SERVA Lightning Red, 100 or 250  $\mu$ l Dimethylformamide (DMF, water-free), 25 ml or 2 x 25 ml 2x SERVA SDS Sample Buffer pH 8.3. Reduction reagents are not included.

#### Ordering Information

| Product             | Quantity    | Cat. No. |
|---------------------|-------------|----------|
| SERVA Lightning Red | 500 lanes   | 43401.01 |
| for 1D SDS PAGE     | 1.250 lanes | 43401.02 |

#### Related Products

| Product                   | Quantity | Cat.-No. |
|---------------------------|----------|----------|
| SERVA HPE™ Lightning Red* | 1 Kit    | 43400.01 |

\* Kit contains 250  $\mu$ g fluorescence dye and 25  $\mu$ l DMF

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