TECHNICAL NOTES | SERVA Electrophoresis

Biochemicals
Electrophoresis
Bioseparation
Life Sciences
Specials

SERVA HPE™ Lightning Red

Fluorescent Pre-labeling of Proteins in 2D PAGE

The fluorescence dye

SERVA HPE™ Lightning Red is a fluorescent dye for rapid labeling proteins prior to 2D 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.

Unaffected by additives and pH value

SERVA HPE™ Lightning Red is compatible with all additives typically used for sample solubilisation and protein extraction, including carrier ampholytes (sometimes also called “IPG buffers”) and reductants like dithiothreitol (DTT) and dithioerythreitol (DTE). Alkalescent conditions are sufficient, it is not required to titrate the pH to a defined value. This is in contrast to other fluorescent dyes used for labeling lysine that are affected by additives as mentioned above as well as by pH conditions of the protein sample.

SERVA HPE™ Lightning Red has a number of advantages:

- Direct detection
- No staining and washing steps after the run
- Very high sensitivity, < 100 pg protein / spot
- Wide dynamic and linear range of > 10^4
- No over-staining effects
- Fully MS compatible
- After imaging gel can be further processed by Western blotting.

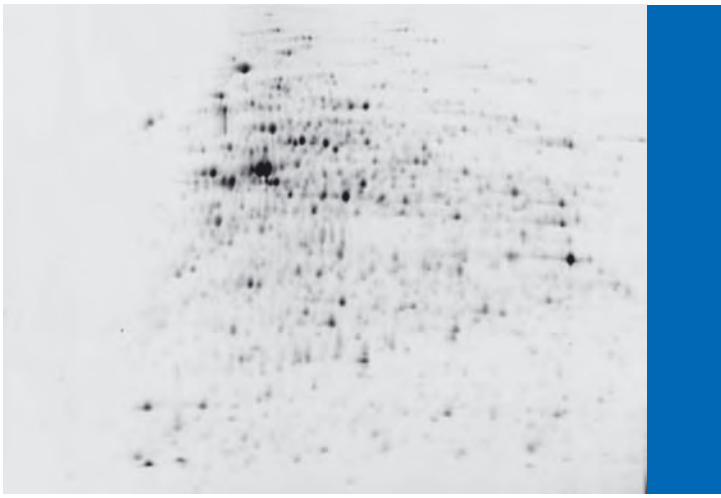

Fig. 1: 50 µg extract of pre-stained *B. subtilis* lysate in a SERVA IPG *Blue*Strip (18 cm, pH 4-7) followed by SDS PAGE in a SERVA 2D HPE Large Gel 12.5 % flatbed gel.

With kind permission by Knut Büttner, Institute for Microbiology, University of Greifswald, Germany.

Easy workflow

The labeling procedure is simple and quick and does not require any additional measures like clean-up or centrifugation steps. When electrophoresis is completed no extra treatment is neccessary for detection.

No detectable change in electrophoretic mobility

The isoelectric point of the labeled protein does not differ from the pI of the non-labeled protein, because each dye molecule carries one intrinsic positive charge. The shift of the electrophoretic mobility in the second dimension, the SDS electrophoresis step, is very low; migration differences between labeled and non-labeled 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. Therefore patterns of two-dimensional electrophoresis separations of labeled and non-labeled protein mixtures do not show any detectable differences.

SERVA HPE™ Lightning Red compared to silver staining

Traditionally silver staining of 2D gels is preferred to other staining methods when highest sensitivity of detection is required. However silver stained spots exhibit very quickly saturation, therefore quantification of spots is not feasible. When directly compared in the identical gel (see Fig. 2) silver staining shows a high number of proteins with much higher intensity, however the scan of the fluorescent label displays a higher spot number without limitation in quantification.

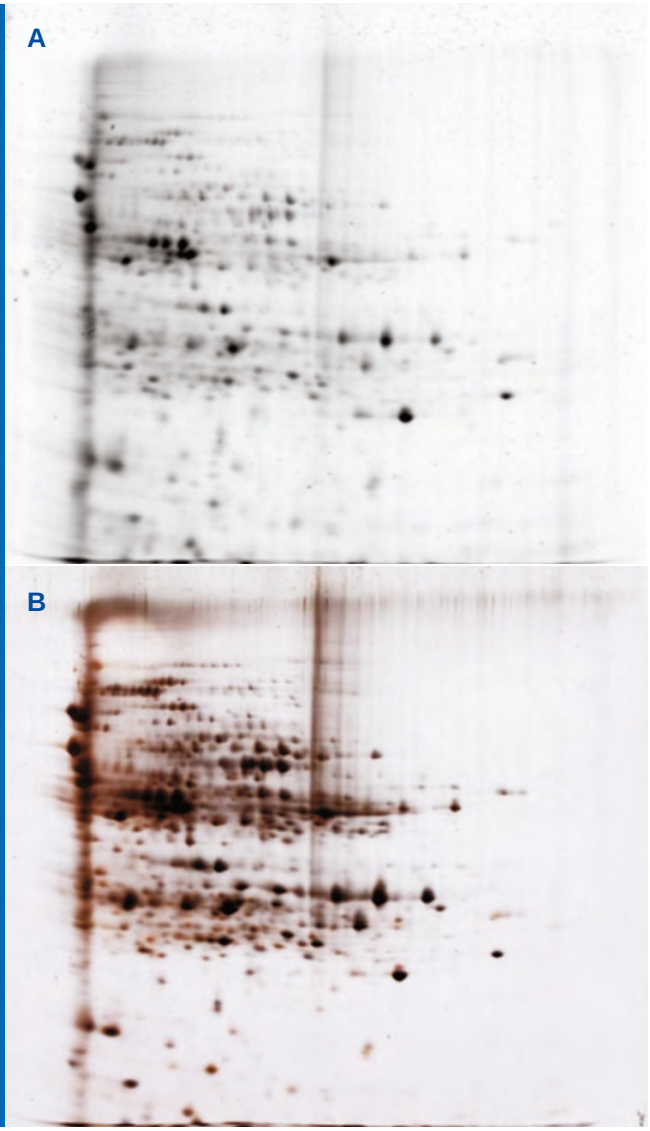

Fig. 2: 20 µg extract of pre-stained *E. coli* lysate in a SERVA IPG *Blue*Strip (7cm, pH 5-8) followed by SDS PAGE in a SERVA*Gel*™ TG PRiME™ 14 % vertical mini gel. A: fluorescent scan with typhoon imager; B: post-stained with highly sensitive SERVA HPE™ Silver Staining (over-stained, many spots saturated).

How it works

The fluorescence dye binds to primary amino groups, e. g. the ε-amine of the lysine residues in proteins and peptides. For standard labeling 1 µg protein is labeled with 80 pmol SERVA HPE™ Lightning Red. To enhance detection sensitivity the dye/protein ratio can be further increased without negative effects. Detection of labeled 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, allowing detection of proteins down to 100 pg protein per spot.

If spots have to be picked 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.

Labeling procedure

1. Dissolve 250 µg of SERVA HPE™ Lightning Red in 25 µl water-free dimethylformamide; the fluorescence dye solution is stable for at least 6 months when stored at -20 °C.
2. Proteins must be dissolved in denaturing lysis buffer.
3. Typically label 1 µg protein with 80 pmol SERVA HPE™ Lightning Red: e.g. add 1 µl of dye solution to 30 µl protein solution (10 µg protein / µl lysis buffer).
4. Mix gently and place vial at 0 °C for 15 min.
5. Apply labeled protein solution on IPG strip via cup- or rehydration loading.

Ordering Information

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

* Kit contains 250 µg fluoresence dye and 25 µl DMF

SERVA Electrophoresis
SERVA Electrophoresis GmbH
Carl-Benz-Str. 7 · D-69115 Heidelberg · Germany
Phone: +49 (0) 6221 13840-0 · Fax: +49 (0) 6221 13840-10
E-Mail: info@serva.de · Internet: www.serva.de