SERVA

SERVA PRiME™ Lightning Red

Direct fluorescence pre-labeling of proteins for SDS PAGE

Content

SERVA PRiME™ Lightning Red

1. General Information

SERVA PRiME™ Lightning Red is a fluorescent dye for rapid labeling 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. If required, subsequent silver staining is also possible.

Advantages

- Direct detection
- No staining and washing steps after the run
- Very high sensitivity ($\leq$ 0.4 ng transferrin)
- Wide dynamic and linear range
- No over-staining effects
- Fully MS compatible
- Gel can be further processed by Western Blotting

1.1. Chemical compatibility

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

1.2. 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.

1.3. Compared to silver and Coomassie® staining

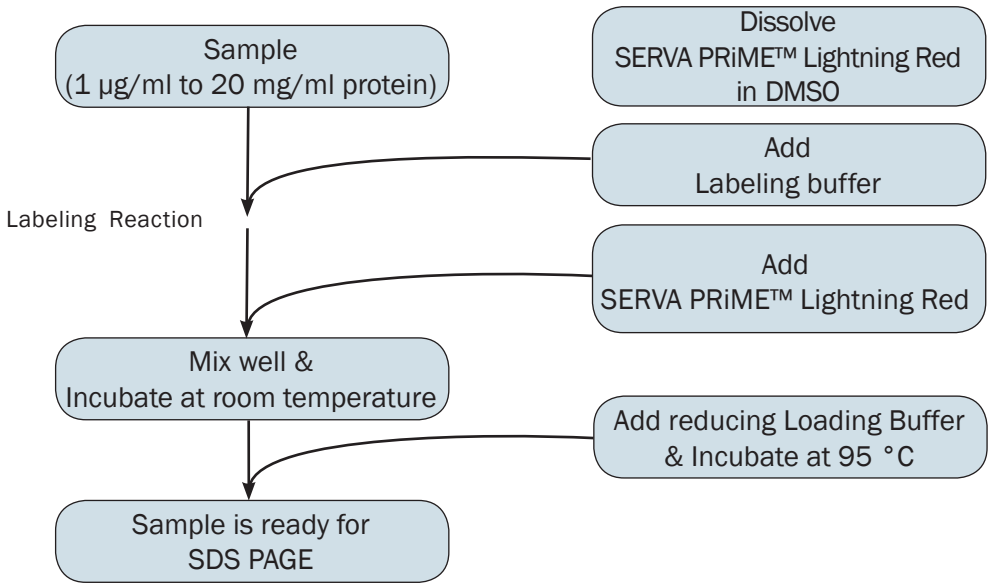
Traditionally, silver staining of PAGE gels is preferred to other staining methods when highest sensitivity of detection is required. However silver stained bands exhibit very quick saturation, therefore quantification of proteins is not feasible.
When gels are compared with the same sample load, silver staining shows bands with higher intensity, however the scan of the fluorescent label displays the bands without limitation in quantification. Compared to SERVA PRiME™ Lightning Red and to silver stained gels Coomassie staining is less sensitive.

Easy pre-labeling procedure combined with high sensitivity and excellent quantification properties make SERVA PRiME™ Lightning Red to the fluorescent stain of choice in SDS PAGE gel staining.

1.4. Quick and easy labeling procedure

When electrophoresis is completed no extra treatment is necessary for detection. Samples of protein concentrations between 1 µg/ml and 20 mg/ml can be used and no purification or concentration steps after labeling are necessary.

Labeling flowchart



1.5. Labeling and fluorescence features

The fluorescence dye binds to primary amino groups, e. g. the ε-amino groups of the lysine residues in proteins and peptides. 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.

The fluorescence quantum yield (QY) is defined as the ratio of the number of photons emitted to the number of photons absorbed.

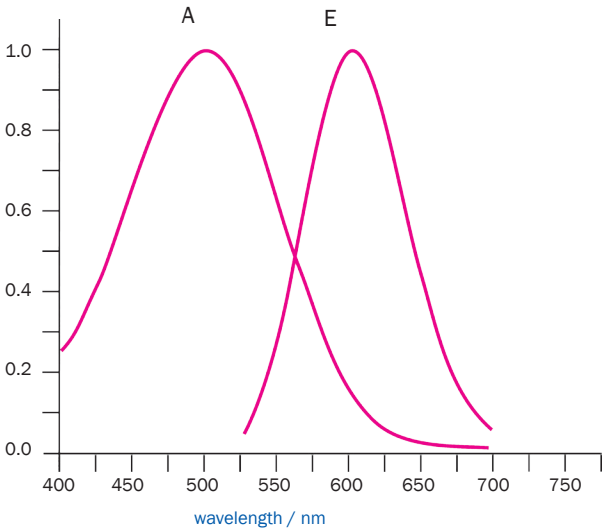


Fig. 1: Absorption- (A) and emission spectrum (E) of protein bound SERVA Lightning Red

1.6. Protein fixation for subsequent mass spectrometry (MS)

If bands must be cut from the gel for mass spectrometry analysis the gels could be fixed in acetic or citric acid and alcohol without any losses in signal intensity for at least 10 days.

1.7. Components und storage conditions

Component	Size	Storage temperature
SERVA PRiME™ Lightning Red	1 vial	+ 2 °C bis + 8 °C (light protected)
DMSO, water-free	250 µl	+ 2 °C bis + 8 °C
Labeling Buffer	10 ml	+ 2 °C bis + 8 °C
2x Loading Buffer	10 ml	+ 2 °C bis + 8 °C

In water-free DMSO reconstituted dye will be stable for at least 6 months if stored in aliquots at -20 °C.

2. Labeling Procedure

2.1. Quantitative and qualitative labeling

The protein labeling can be done either qualitative or quantitative.

Required solutions:

- SERVA PRiME™ Lightning Red: To reconstitue the dye add 250 µl DMSO in the vial
- 1 M DTT solution: 154 mg DTT add 1 ml dist. water
- Reducing Loading Buffer: 500 µl 2x Loading Buffer + 50 µl 1 M DTT

	Qualitative protocol	Quantitative protocol
Sample (1 µg/ml to 20 mg/ml)	2 µl	
Labeling Buffer	+ 17 µl	
SERVA PRiME™ Lightning Red	+ 1 µl	
	Mix	
Incubation at room temperature	3 - 5 min	30 min
Reducing Loading Buffer	+ 20 µl	
	Mix	
Incubation at 95 °C	5 min	

Important:

2x Tris-Glycine SDS PAGE Sample Buffer can be used alternatively.

Then, the labeling buffer must be 100 mM Tris-HCl, pH 8.5 with 0.5 % (w/v) SDS.

Now, the labeled sample is ready for SDS PAGE.

Please note:

The labeling protocol can also be used for acidic samples, e.g. urine. The protein concentration of urine samples should be adjusted to 20 µg/ml.

2.2. Protein labeling of samples in lysis buffer

IMPORTANT:

It is not possible to mix lysis and labeling buffer due to precipitation. Therefore, labeling will be performed in lysis buffer.

After the labeling reaction, the proteins are precipitated, e.g. according to the protocol of Wessel and Fluegge (see appendix). Then, the sample can be dissolved in the sample buffer of choice.

3. Detection Limit, Linearity and Dynamic Range

3.1. Time dependency of transferrin labeling

- Protein concentration: 20 mg/ml
- Incubation time of labeling reaction: 0 – 30 min
- Detection: SERVA BlueImager
- Exposure time: 1 s

3.2. Detection limit and linearity: Labeling of transferrin

- Protein concentration: 20 mg/ml
- Labeling according long protocol: 30 min incubation
- Preparation of a dilution series: 20 µg/ml to 10 ng/ml
- Applied sample volume per lane: 5 µl

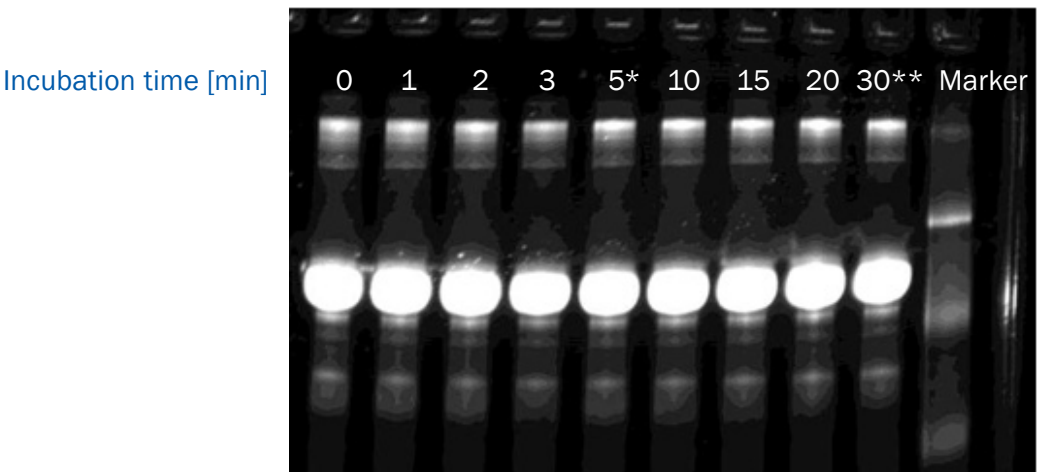


Fig. 2: SERVAGel™ TG PRiME™ 10 % pre-cast gel with Lightning Red labeled Transferrin samples (20 mg protein /ml)

* Incubation time quantitative protocol

** Incubation time qualitative protocol

3.2.1. Detection using SERVA BlueImager

- Exposure time: 15 s
- Detection limit: ≥ 200 pg
- Analysis: Kapelan LabImage 1D software

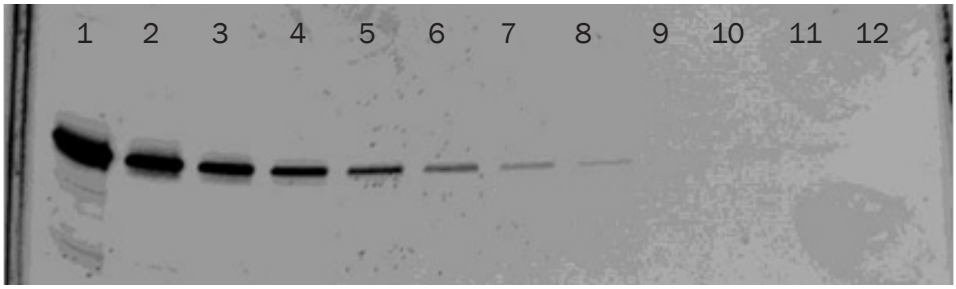


Fig. 3.1.: Detection limit in SERVA*Gel*™ TG PRiME™ 10 % pre-cast gel with dilution series of Lightning Red labeled Transferrin samples (protein amount - lane 1: 100 ng; lane 6: 3.12 ng; lane 12: 50 pg)

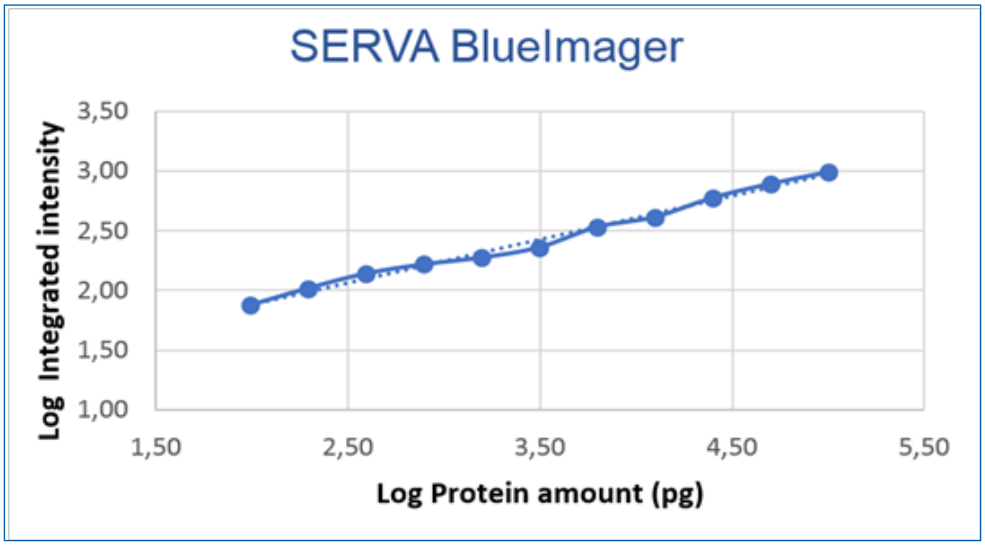


Fig. 3.2.: Analysis of the labeling linearity; detection system: SERVA BlueImager

3.2.2. Detection using BIO-1000F Fluorescence Scanner

- Fluorescence Scanner: 24x
- Detection limit: ≥ 12.5 ng

Fig. 4: Detection limit in SERVA*Gel* ™ TG PRiME™ 10 % pre-cast gel with dilu-tion series of Lightning Red labeled Transferrin samples (protein amount - lane 1: 100 ng; lane 6: 3.12 ng; lane 12: 50 pg)

3.2.3. Detection using SERVA Musketeer

- Exposure time: 5 s
- Iris: 0.95
- Detection limit: ≥ 400 pg
- Analysis: Kapelan LabImage 1D software

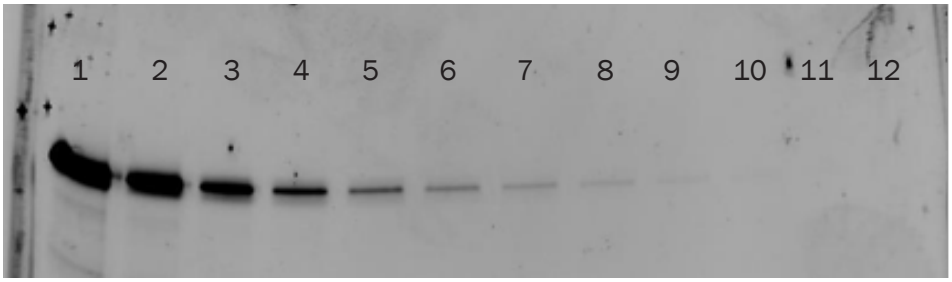


Fig. 5.1.: Detection limit in SERVA*Gel*™ TG PRiME™ 10 % pre-cast gel with dilution series of Lightning Red labeled Transferrin samples (protein amount - lane 1: 100 ng; lane 6: 3.12 ng; lane 12: 50 pg)

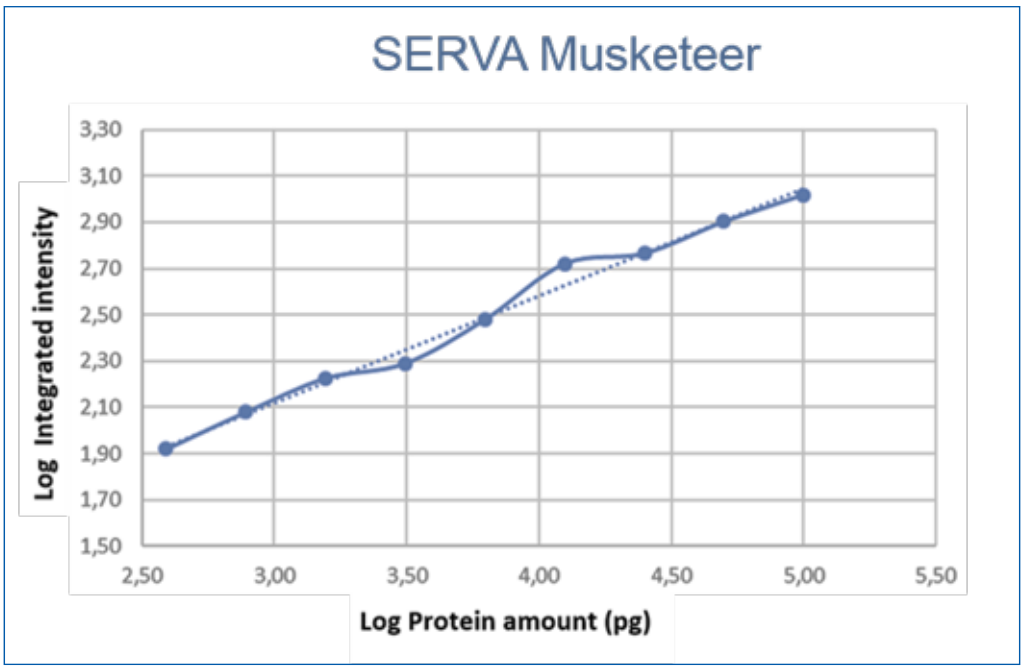


Fig. 5.2.: Analysis of the labeling linearity; detection system: SERVA Musketeer

3.2.4. Detection using GE Healthcare Typhoon Scanner

- Excitation: 532 nm
- Emission: 610 nm (PMT 600)
- Protein concentration: 20 µg/ml (1 µg/ml)
- Detection limit: ≥ 100 pg (≥ 300 pg)
- Analysis: Kapelan LabImage 1D software

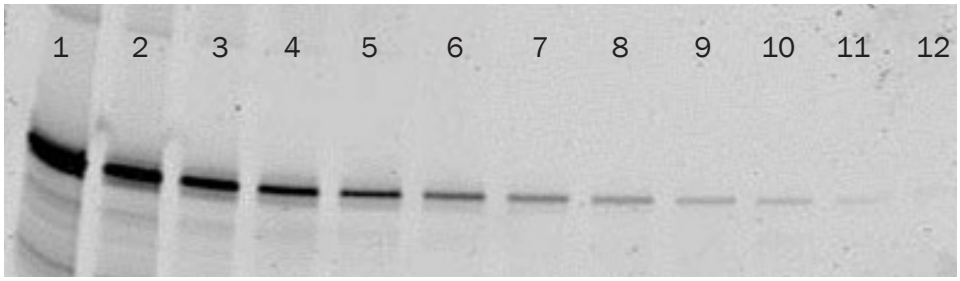


Fig. 6.1.: Detection limit in SERVA*Gel*™ TG PRiME™ 10 % pre-cast gel with dilution series of Lightning Red labeled Transferrin samples (protein amount - lane 1: 100 ng; lane 6: 3.12 ng; lane 12: 50 pg)

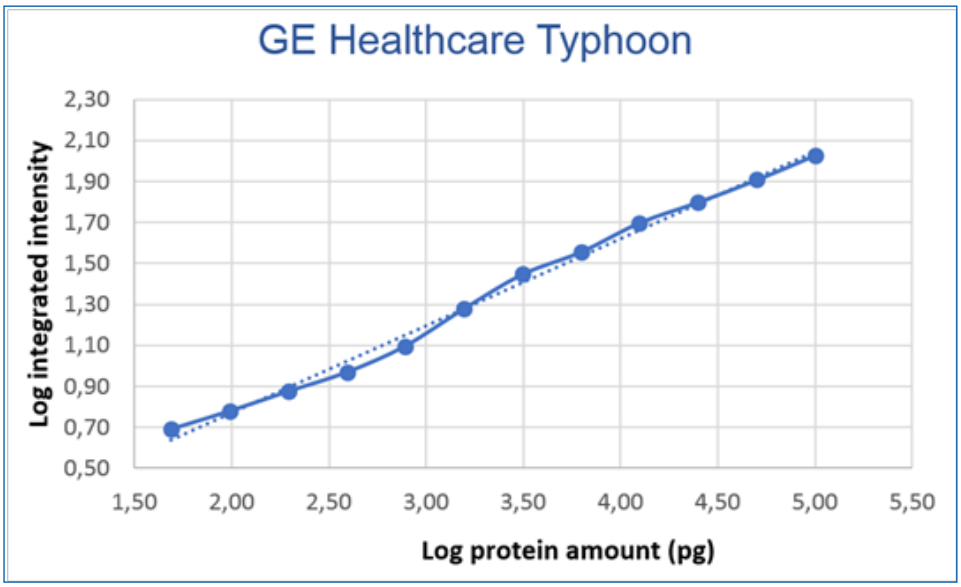


Fig. 6.2.: Analysis of the labeling linearity; detection system: GE Healthcare Typhoon

3.3. Dynamic range

Sample: Ovalbumin (40 µg/ml, labeled with SERVA PRiME™ Lightning Red) spiked with Transferrin (from 5 µg/ml to 5 ng/ml, also labeled with SERVA PRiME™ Lightning Red). With a transferrin: ovalbumin ratio of 1:8200 (lane 11) Transferrin is still detectable.

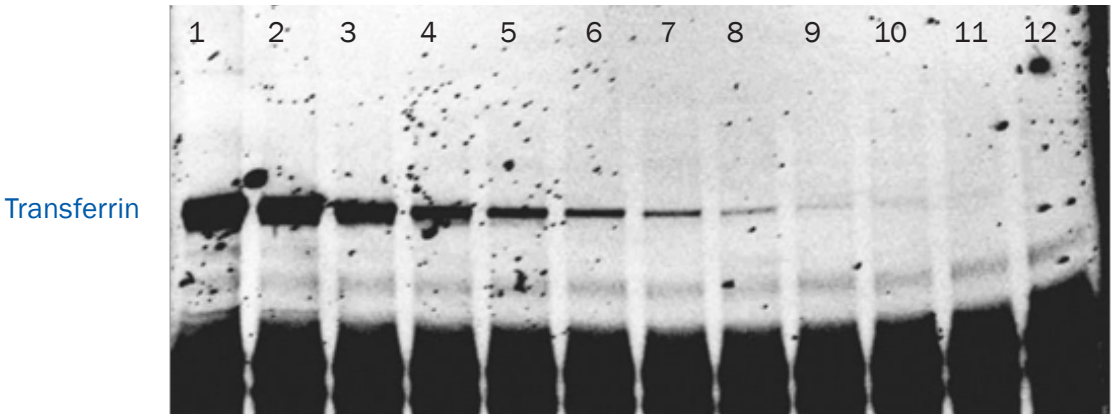


Fig. 7: SERVA*Gel*™ TG PRiME™ 4 - 12 % pre-cast gel of Transferrin-spiked Ovalbumin

4. Western Blotting and Immuno Detection of Labeled Proteins

After electrophoresis, SERVA PRiME™ Lightning Red labeled proteins can be transferred by Western Blotting onto Nitrocellulose (NC)- and PVDF membranes. The membranes should have low fluorescence background.

IMPORTANT:
If Western Blotting is performed, Lightning Red should be diluted 1:10 in Labeling Buffer, to get a minimal labeling (6.25 pmol/µg protein) of the protein sample (20 mg/ml).

4.1. Protein labeling

The labeling procedure does not change despite the dilution of the Lightning Red dye.

Required solutions:

- Dilution of Lightning Red:
- 10 µl SERVA PRiME™ Lightning Red + 90 µl Labeling Buffer
- 1 M DTT solution: 154 mg DTT add 1 ml dist. water
- Reducing Loading Buffer: 500 µl Loading Buffer + 50 µl 1 M DTT

	Qualitative protocol	Quantitative protocol
Sample (1 µg/ml to 20 mg/ml)	2 µl	
Labeling Buffer	+ 17 µl	
SERVA PRiME™ Lightning Red	+ 1 µl	
	Mix	
Incubation at room temperature	3 - 5 min	30 min
Reducing Loading Buffer	+ 20 µl	
	Mix	
Incubation at 95 °C	5 min	

4.2. Protein detection

In the following analysis 5 µg Transferrin are incubated and labeled with different Lightning Red (LR) concentrations (40 to 0.16 ng LR/µg protein).

4.2.1. Detection within the gel

- Sample: 5 µl/lane (equal to 1.67 µg protein)
- Detection: SERVA BlueImager
- Exposure time: 10 s

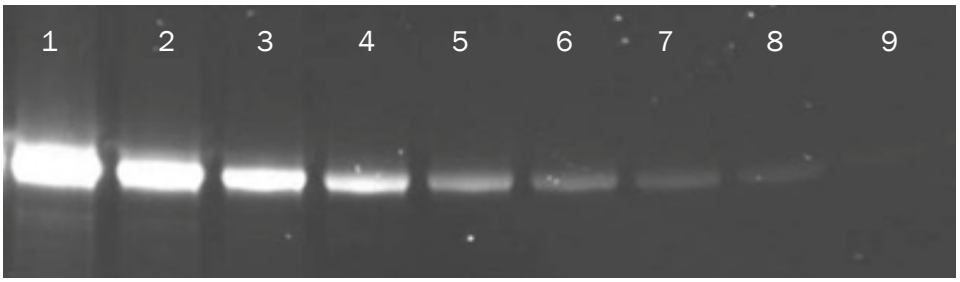


Fig. 8: SERVA*Gel*™ TG PRiME™ 8 % pre-cast gel with dilution series of Lightning Red labeled Transferrin (prior to semi-dry transfer)

4.2.2. Protein detection after transfer on a blot membrane

The Western Blotting was performed with SERVA Xpress PVDF Blotting Kit (cat. no. 42664) according to the instructions provided by the manufacturer.

- Membrane: PVDF, Pore size 0.2 µm
- Detection system: SERVA Musketeer
- Exposure time: 3 s

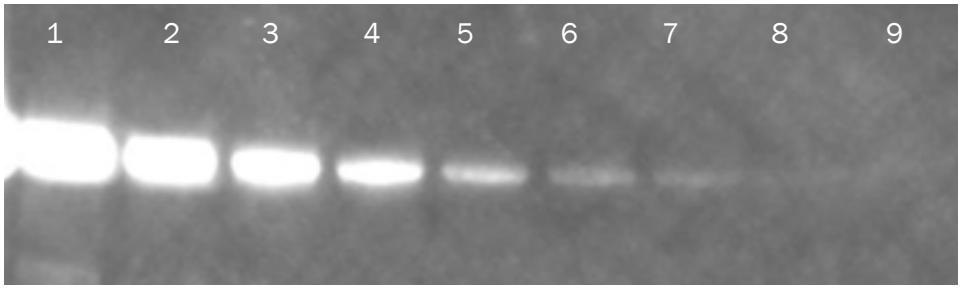


Fig. 9: SERVA*Gel*™ TG PRiME™ 8 % pre-cast gel with dilution series of Lightning Red labeled Transferrin blotted on a PVDF membrane

4.2.3. Immuno chemical analysis and chemiluminescence detection

Step	Duration	Solution
Blocking	1 h	1x SERVA BlueBlock (SERVA cat. no. 42591)
1. Antibody	1 h	Rabbit-Anti human Transferrin (1:50,000 in 1x SERVA BlueBlock)
Wash	3 x 5 min	1x TBS-Tween
2. Antibody	1 h	Anti-rabbit IgG-HRP (1:2.000 in 1x SERVA BlueBlock)
Wash	3 x 5 min	1x TBS-Tween
Detection	Substrate: SERVALight EOS, detection system: GeneGnome	

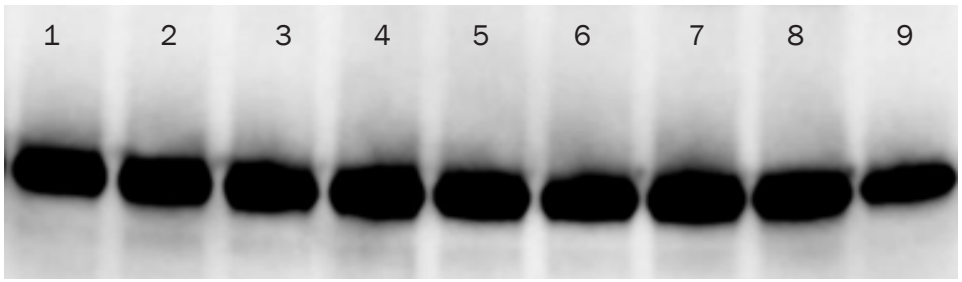


Fig. 10: PVDF membrane with dilution series of Lightning Red labeled Transferrin

Appendix:

Methanol-Chloroform Precipitation of Proteins according to Wessel and Fluegge

(Anal. Biochem. 1984, 138 (1): 141 – 143)

1. General Information

The protein precipitates form at the interphase between the top aqueous layer and the organic (chloroform/methanol) bottom layer. The aqueous layer contains nucleic acids, salts, detergents, reducing agents etc. The organic layer contains lipids. This protocol is suitable for both, protein enrichment and removal of interfering molecules, e.g. lipids and nucleic acids.

2. Required Reagents and Devices

- Chloroform (SERVA cat. no. 45627)
- Methanol (SERVA cat. no. 45631)
- Distilled water (dist. H_2O)
- Suitable sample tubes, e.g. 1.5- or 2.0 ml-tubes
- Vortex
- Microcentrifuge, e.g. SERVA BlueSpin Mini

3. Protocol

Please note: This protocol is for 1.5 ml-tubes. If necessary, scale up is possible.

- Pipette 150 µl of the protein sample (protein content 50 – 300 µg) in a 1.5 ml-tube. To make up the volume of 150 µl, you may add the required amount of sample buffer (Same buffer which was used to prepare the protein sample).
- Add 600 µl methanol and 150 µl chloroform and mix thoroughly by vortexing.
- Add 450 µl dist. H_2O and mix well by vortexing.
- Centrifugation: 5 min at min. 12,300 x g
- Remove the upper aqueous phase carefully, without disturbing the white protein precipitate formed between the two phases.
- Add 450 µl methanol and mix well by vortexing.
- Pellet the protein by centrifugation: 5 min at min. 12,300 x g
- Remove the supernatant completely. Be careful not to lose the pellet.
- For easier visibility, mark the position on the outside of the tube.
- Centrifuge again (5 min at min. 12,300 xg) to collect residual chloroform/methanol at the bottom of the tube. Remove the supernatant carefully.
- Air-dry the protein pellet. Alternatively use a speed-vac for approx. 10 min.

IMPORTANT:
The pellet should not be completely dry because dissolving will be easier afterwards. Traces of chloroform and methanol may interfere with fluorescence labeling.

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