INSTRUCTION MANUAL

SERVA ProteinStain Fluo-R

Fluorescent dye for protein detection

(Cat. No. 35090; 35091)

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1. General information

SERVA ProteinStain Fluo-R is a highly sensitive fluorescent dye for detection of proteins in e. g. SDS-PAGE, native PAGE or 2D gels. The dye does not interfere with immunodetection. Therefore prior to Western Blotting it is possible to stain the gel with SERVA ProteinStain Fluo-R. It is as sensitive as silver staining. The dye has a good linearity, high contrast and is compatible with MS/MS analysis. The staining can be as well combined with silver staining and DIGE. The dye can be excited with UV light (254 nm) as well as light of wave length 473/488 nm. Excitation with laser light of wave length 532 nm is as well possible, but less sensitive. Detection is performed at 610 nm.

1.1. Preparation of staining solution

Dissolve 5 mg SERVA ProteinStain Fluo-R per 3 L dist. H_2O to receive 1 µM staining solution.
SERVA ProteinStain Fluo-R solution is supplied as 1 mM solution. Dilute the dye solution 1: 1,000 to get a 1µM staining solution.

1.2. Preparation of stock solution

20 mM stock solution: 5 mg per 150 µl water

1.3. Storage

Cat. No.	Product	Temperature
35090.01	SERVA ProteinStain Fluo-R	+15 - +30 °C
35090.02	SERVA ProteinStain Fluo-R	+15 - +30 °C
35091.01	SERVA ProteinStain Fluo-R solution	+2 - +8 °C

2. Staining protocols

2.1. Protocol for fast staining of mini gels

Step	Incubation time	Solution
1. Staining	1 h	1 µM Fluo-R in 40 % (v/v) Ethanol; 10 % (v/v) Acetic acid
2. Destaining	20 min	40 % (v/v) Ethanol; 10 % (v/v) Acetic acid
3. Wash	10 min	dist. H_2O
4. Documentation		

2.2. Staining protocol for large gels

Step	Incubation time	Solution
1. Fixing	Overnight	30 % (v/v) Ethanol; 10 % (v/v) Acetic acid
2. Wash	4 x 30 min	20 % (v/v) Ethanol
3. Staining	6 h	1 µM Fluo-R
4. Equilibration	2 x 10 min	dist. H_2O
5. Destaining	15 h	40 % (v/v) Ethanol; 10 % (v/v) Acetic acid
6. Equilibration	2 x 10 min	dist. H_2O
7. Documentation		

2.3. Direct staining protocol (Fluo-R as sample buffer component)

Step	Incubation time	Solution
1. Preparation of sample buffer		Add 1 µl of 20 mM stock solution Fluo-R to 1.5 ml sample buffer
*** Avoid dyes like bromophenol blue and/or chelating agents, e. g. EDTA in the sample buffer.**		
2. Electrophoresis	Time according to standard procedure	According to standard procedure
3. Destaining	20 min	40 % (v/v) Ethanol; 10 % (v/v) Acetic acid
4. Equilibration	10 min	dist. H_2O
5. Documentation		

3. Subsequent procedures for Fluo-R stained gels

3.1. Western blotting

After staining the gel with SERVA ProteinStain Fluo-R (see section 2.), subsequent western blotting is possible.
The gel and the membrane are equilibrated for 30 min with blotting buffer. Thereafter blotting and immuno detection can be performed according to standard procedure.

3.2. Silver stain

Silver staining (according to standard procedure) of SERVA ProteinStain Fluo-R stained gels can be performed after 30 min fixation in mit 45 % (v/v) methanol/10 % (v/v) acetic acid.