INSTRUCTION MANUAL

SERVA*Light* Vega

Western Blot Chemiluminescence HRP Substrate Kit

(Cat. No. 42588)

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1. SERVA*Light* Vega

1.1. General information

SERVA*Light* Vega is an extremely sensitive enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) conjugates on immunoblots. The extremely intense signal output enables detection of **low picogram** (10^{-12}) amounts of antigen. The sensitivity, intensity and duration of the signal allow easy detection of HRP using imaging equipment.

Advantages:

- Compatible with other chemiluminescence detaction protcols, e.g. ECL Amesham, ECL Pierce.
- Detection with films or digitally by using documentation systems suitable for chemiluminescence, e.g. ProXima C16Phi+, Isogen
- Very short exposure times and/or highly diluted antibody solution because of extremely intense light output

SERVA*Light* Vega is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

SERVA*Light* is produced by Cyanagen Srl. Cyanagen Srl is subject of US and EU patent application number US7803573; EP1962095; US7855287; EP1950207, together with other equivalent granted patents and patent applications in other countries.

1.2. Kit components

Cat. No.	SERVA*Light* Vega Luminol Solution	SERVA*Light* Vega Peroxide Solution
42588.01	25 ml	25 ml
42588.02	125 ml	125 ml
42588.03	250 ml	250 ml

1.3. Storage conditions

Upon arrival store the kit at 2 – 8 °C.
If stored at the recommended temperature at least usable until: see expiry date on package.

2. Detection procedure

2.1. Important before starting

Safety information:
For safety reasons always wear suitable protective gloves and clothing, when you work in the lab.

It is essential to optimize all components of the system including sample amount, primary and secondary antibody concentration, and the choice of membrane as well as blocking reagents. Therefore, the recommended antibody dilutions has to be critically adapted. Due to this high sensitivity, the substrate requires fewer amounts of sample, primary and secondary antibodies than most commercially available substrates.

- The antibody solutions required will be much more dilute than those used with colorimetric HRP systems. To optimize the appropriate concentrations, perform a systematic dot blot analysis.
- No blocking reagent is optimal for all systems Therefore, it is important to determine the appropriate blocking buffer for each Western blot system. Determining the proper blocking buffer can increase sensitivity and prevent non-specific signal caused by cross-reactivity between the antibody and the blocking reagent. Furthermore, when switching from one substrate to another, a diminished signal or increased background can result if the blocking buffer is not optimal for the new system.
- Use a sufficient volume of wash buffer, blocking buffer, antibody solution and the substrate working solution to cover blot and ensure that it never becomes dry. Large blocking and wash buffer volumes result in reduced non-specific signal.
- Add Tween®-20 (final concentration of 0.05%) to the blocking buffer and when preparing all antibody dilutions to reduce non-specific signal. Use only high-quality products guaranteed to be low in peroxides and other contaminants.
- Do not use milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin.
- For optimal results, use a shaking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.

- Do not handle membrane with bare hands. Always wear gloves or use clean forceps. All equipment must be clean and free of foreign material. Metallic devices, e.g. scissors must have no visible signs of rust. Rust may cause speckling and/or high background
- **SERVA*Light* Vega** substrate working solution is stable for 5 day at room temperature. Exposure to the sun or any other intense light can harm the working solution. For best results keep the working solution in an amber bottle and do not expose to any intense light. Short-term exposure to typical laboratory lighting will not harm the working solution.

2.2. Required, not supplied materials and solutions

- **Western-Blot-Membran:** Use any suitable protocol to separate proteins by electrophoresis and transfer them to a nitrocellulose membrane.
- **Dilution buffer:**
  TBS (Tris Buffered Saline) or PBS (Phosphate Buffered Salin)
- **Blocking buffer:**
  Suitable blocking in TBS oder PBS
- **Wash buffer (TBS-T or PBS-T):**

  5 ml 10 % (v/v) Tween®-20
  ad 1 l TBS or PBS
- **Blocking reagent:**

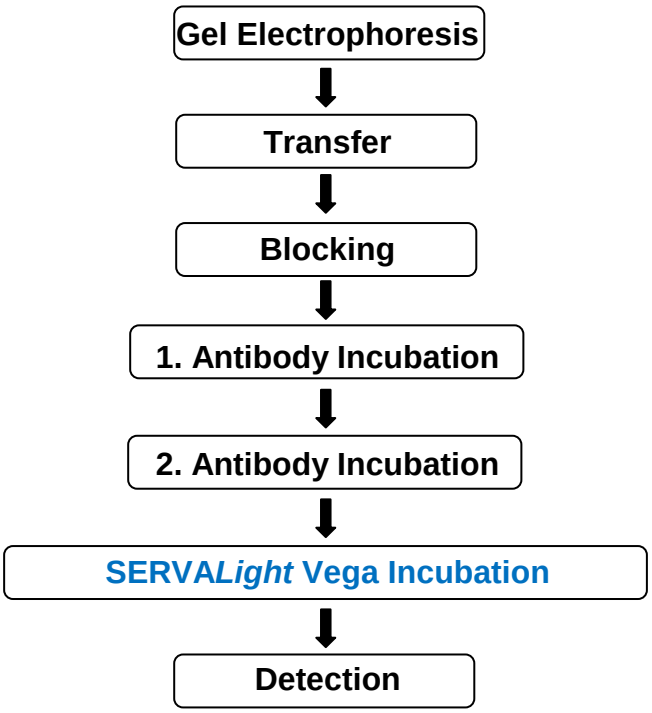
  0.5 ml 10 % (v/v) Tween®-20
  ad 100 ml blocking buffer (with the same base components as the dilution buffer)
- **Primary antibody (1. Ab, target protein specific antibody):**
  - Stock solution in dilution buffer: 1 mg/ml
  - Working solution: 1: 100 – 1: 5,000 dilution in blocking reagent.

  The necessary dilution to use depends on the specific primary antibody and the amount of antigen on the membrane and will require optimization for each experimental system.
- **Secondary antibody HRP-labelled (2. Ab, specific for primary antibody):**
  - Stock solution in blocking reagent: 1 mg/ml
  - Working solution: 1: 1,000 – 1: 15,000 dilution in blocking reagent.

  The optimal dilution varies depending on the HRP conjugate and the amount of antigen on the membrane.

- Rotary platform shaker: For agitation of membrane during incubations.
- Film cassette, developing and fixing reagents:
  For processing auto-radiographic film.

2.3. Overview of the Western Blot procedure



2.4. Protocol

(1) Remove blot from the transfer apparatus and block non-specific sites with blocking reagent for 30 - 60 min at room temperature (RT) with shaking. For best results, block for 1 h at RT or overnight at 2 – 8 °C.

(2) Remove the blocking reagent and add the appropriate primary antibody dilution. Incubate blot for 1 – 2 h with shaking or overnight at 2 - 8 °C.

(3) Wash membrane by suspending it in wash buffer and agitating for 5 min. Replace wash buffer at least 4 - 6 times. Increasing the wash buffer volume and/or the number of washes may help reduce background. Briefly rinsing membrane in wash buffer before incubation will increase wash efficiency.

(4) Add the appropriate HRP conjugate dilution. Incubate blot for 30 – 60 min at RT with shaking. Increasing incubation time will lead to higher background.

(5) Repeat Step 3 to remove unbound HRP conjugate.
Note: Membrane must be thoroughly washed after incubation with the HRP conjugate.

(6) Allow the detection solutions to equlibrate to 20 - 25°C temperature before opening.

(7) Prepare the substrate working solution by mixing equal parts of the peroxide solution and the luminol solution. Use 0.1 ml of working solution per cm^2 of membrane.

(8) The working solution is stable for 5 days at 2 - 8 °C.

(9) Incubate blot with the substrate working solution for 1 min.

(10) Place the protected blot (e.g. air-bubble-free in saran wrap) in a film cassette with the protein side facing up. Turn off all lights except those appropriate for film exposure (e.g. a red light). Film must remain dry during exposure. For optimal results, perform the following precautions: make sure, excess substrate is removed from the blot and the membrane protector. Use gloves during the entire film-handling process. Never place a blot on developed film. There may be chemicals on the film that will reduce signal.

(11) Carefully place film on top of the protected blot.

(12) A recommended exposure time to start with is 30 s, however, exposure time can be varied, e.g. 15 s, 1 min, 5 min, to achieve optimal results. Enhanced or pre-flashed auto-radiographic film is not necessary.
Caution: Light emission is intense and any movement between the film and the blot can cause artefacts on the film.

(13) Develop film using appropriate developing solution and fixative.

(14) Blot may be stripped and re-probed if necessary.

3. Trouble shooting

Problem	Possible cause	Countermeasure
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly	Higher dilution of HRP conjugate
	Insufficient quantities of antigen or antibody	Increase amount of antigen or antibody
	Inefficient protein transfer	Optimize transfer
	Reduction of HRP or substrate activity	Use fresh reagents
Blot glows in the dark	Too much HRP in the system depleted the substrate	Higher dilution of HRP conjugate
Membrane has brown bands		
Reverse image on film		
High background	Too much HRP in the system depleted the substrate	Higher dilution of HRP conjugate
	Inadequate blocking or inappropriate blocking reagent	Optimize blocking conditions and reagent
	Film over exposed	Reduce exposure time
	Too much antigen or antibody	Optimize amount of antigen or antibody
	Inadequate washing	Increase length, number and volume of washing steps
Speckled background	Formation of aggregates in HRP conjugate	Filter through 0.2 µm filter
Spots within protein bands	Inefficient protein transfer	Optimize transfer
	Unevenly hydrated membrane	Hydrate the membrane properly
	Bubbles between film and membrane	Remove all air bubbles before exposure to film
	Too much antigen or antibody	Optimize amount of antigen or antibody
	Inadequate washing	Increase length, number and volume of washing steps
Non-specific bands	Short signal duration and high background (too much HRP)	Higher dilution of HRP conjugate
	Signal duration and background OK (too much primary antibody)	Higher dilution of primary antibody
	SDS present in the immunoassay	Do not use SDS

4. Ordering information

Membrans	**Cat. No.**
Immobilon™ (PVDF), 26.5 cm x 3.75 m, Pore size: 0.2 µm (1 roll)	42574
Fluorobind (PVDF), 10 x 10 cm, Pore size: 0.2 µm (20 sheets)	42573
Fluorobind (PVDF), 25 cm x 3 m, Pore size: 0.2 µm (1 roll)	42571
Protein Standard	
SERVA Western Blot Protein Standard	39256
Reagents	
Tween® 20	37470
Detection reagents/-kits	
Chemiluminescence Reagent for Horseradish Peroxidase	42582
SERVA*Light* Polaris CL HRP WB Substrate Kit	42584
SERVA*Light* Eos CL HRP WB Substrate Kit	42585
SERVA*Light* EosUltra CL HRP WB Substrate Kit	42586
SERVA*Light* Helios CL HRP WB Substrate Kit	42587

Immobilon™: Trademark of Millipore