Instruction Manual

Chemiluminescence reagent for HRP

Cat. No.: 42582

PRODUCT DESCRIPTION:

The chemiluminescence reagent is a ready-to-use detection solution to which prior to use 30 % hydrogen peroxide (H_2O_2, not included) in a ratio of 1:1000 must be added. This substrate solution can be used for the detection of horseradish peroxidase (HRP)-labelled immobilized proteins (Western-Blot) or nucleic acids (Southern-/Northern-Blot) on membranes.
In the presence of H_2O_2, HRP catalyzes the oxidation of luminol. Immediately following the oxidation, the luminol reaches an energetic excited state and forms an intermediate reaction product which emits light by reaching the ener-getic ground state.
This method allows the detection of membrane bound specific antigens or nucleic acid fragments directly, if labeled with HRP, or indirectly with HRP-labelled antibodies or streptavidin.

Advantages of this method:

- High sensitive, non-radioactive
- Documentation on film or digital by using documentation systems suitable for chemiluminescence, e.g. Proxima, Isogen.
- Detection may be achieved in short exposure times (minutes)
- High resolution

Detection:

- Mix Chemiluminescence Reagent and 30 % H_2O_2 1:1000 to get the detection solution.
- Drain excess buffer from the washed blots. Do not let the mebrane dry out.
- Add the detection solution directly to the blot (0.1 ml/cm^2) and incubate for 1-2 minutes at room temperature.
- Drain off excess detection solution and wrap the membrane in saran foil. Gently remove air bubbles.
- Place the blot fornt side up in the film cassette, place sheet of film on the blot and expose 30-60 sec.
- Develop film
- Depending on the signal intensity choose a longer or shorter exposure time for the second film
- If signal intensity is too high, wait up 20-30 minutes before re-exposing.

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SERVA Electrophoresis GmbH • D-69115 Heidelberg • Carl-Benz-Str. 7
Tel.: +49(0)6221 / 138 40-0 • Fax· +49(0)6221 / 138 40-10 • E-Mail: info@serva.de
http://www.serva.de