Instructions for use

ROTI®Lumin

Chemiluminescent substrate for Horseradish Peroxidase (HRP)-labelled reporter molecules

I. Description and Applications

ROTI®Lumin is a luminol-based chemiluminescent substrate designed for use with peroxidase-labelled (HRP) reporter molecules.
ROTI®Lumin provides good sensitivity over chromogenic substrates in both blotting and microwell assays.
Positive reaction sites are rapidly detected with high sensitivity and minimal background. The use of ROTI®Lumin allows for multiple stripping and reprobing of blots. In blotting applications, permanent results are recorded on X-ray film.
In microwell assays, positive reactions are rapidly detected and read in a luminometer.
ROTI®Lumin can be used in applications such as ELISA, Western blotting, Southern blotting, dot blotting und colony hybridizations.

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The company is a limited partnership with headquarters in Karlsruhe, reg. court Mannheim HRA 100055. Roth Chemie GmbH, with headquarters in Karlsruhe, reg. court Mannheim HRB 100428, is the personally liable partner. Managing Director: André Houdelet. Sales tax identification number: DE 143621073.

II. Principle

In the presence of hydrogen peroxide, HRP converts luminol to an excited intermediate dianion. This dianion emits light on return to its ground state. After reaction with HRP-conjugate, the light emission from ROTI®Lumin reaches maximum intensity within 5 minutes and is sustained for approximately 1-2 hours.

III. Membranes

ROTI®Lumin can be used with nitrocellulose (NC), nylon, and PVDF membranes.
ROTI®PVDF (T830.1), ROTI®Nylon plus (K058.1) and ROTI®NC membranes (HP40.1) are recommended for use with ROTI®Lumin.

IV. Bocking reagents

Milk or casein-based blocking solutions are recommended for use with ROTI®Lumin. BSA or serum-based blocking agents may cause elevated background. For Western-Blotting applications we recommend ROTI®Block (A151.1), an optimised polymer-based blocking reagent.

V. Application

- Perform standard blotting and/or immunoassay procedures.
- Following incubation with HRP antibody or HRP streptavidin, perform at least three washes.
- Mix ROTI®Lumin 1 (P079.1) and ROTI®Lumin 2 (P080.1) in equal volumes. Warm this ROTI®Lumin working solution to room temperature before use. (Stock solutions should be put back to cooling at once.) ROTI®Lumin working solution ist stable for up to one hour at room temperature or up to 24 hours when stored at
  2 °C - 8 °C. Working solution needs not be protected from light.

ELISA Procedure
Add 100 µl / well ROTI®Lumin working solution.
Read in a luminometer with 1 second integration time per well. ROTI®Lumin provides consistent results when read 5-45 minutes after addition of substrate.

Western Blotting Procedure
Use 1 ml substrate / 10 cm^2 membrane

- Immerse membrane in ROTI®Lumin working solution for one minute. This incubation may be performed in the light.
- Remove membrane from ROTI®Lumin working solution, touch the edge of the membrane to a piece of filter paper to remove excess substrate.
- Place membrane between two sheets of plastic foil.
- Expose membrane to X-ray film 1-10 minutes. Light emission begins immediately upon removal from substrate and continues for approximately 2 hours.
- Develop film.

VI. Repeated use of a blot: Removing bound antibodies and probes

For Western-Blots:

- Wash membrane in TBST-buffer
- Incubate in ROTI®Free Stripping Buffer for 30 minutes at 56 °C (e.g. in a water bath with shaking device) in fume hood.
- Wash twice in TBST for 20 minutes each.
- Blocking of the membrane (see IV.)
- Incubation in primary antibody and further detection according to protocol.

Efficiency of stripping may be controlled with brief chemoluminescence staining after washing in TBST. A slight reduction of signals of membrane-bound proteins and an increase in background may occur after repeated stripping.

Please note:
By using ROTI®Block the saturation of nonspecific binding sites will remain stable. No additional blocking is required.

For Southern-Blots:

- Rinse membrane for 5 minutes in Wasing buffer.
- Cover membrane with stripping buffer and incubate under gentle shaking at 55 °C for 20 min.
- Rinse membrane twice for 5 minutes each in 2x SSC.
- Prehybridize membrane before reprobing.

VII. Additionally Required Reagents

Washing Buffer: (example)
100 mM Tris, pH 7.5; 150 mM NaCl, 0.5 % Tween 20.

Stripping Buffer:
For Western-Blots: ROTI®Free Stripping Buffer (Art. No. 0083.1, 3337, 3319)
For Southern-Blots: (prewarm to 55 °C)
0.2 N NaOH, 0.1 % SDS

SSC: ROTI®Stock 20x SSC (Art. No. 1054.1)
TBST: ROTI®Stock 10x TBST (Art. No. 1061.1)
PBST: ROTI®Stock 10x PBST (Art. No. 1059.1)
Blocking: ROTI®Block (Art. No. A151.1)
Casein (Art. No. 8569.1)
Powdered milk (Art. No. T145.1)

VIII. Trouble Shooting

Blotting

Excess signal or high background	• Decrease film exposure time • Decrease HRP conjugate concentration • Increase blocking times • Increase washing times • Load less protein/DNA onto gel
No signal	• Verify transfer by staining protein gel with Coomassie blue or DNA gel with ethidium bromide • Verify protein transfer by staining membrane with Ponceau S or Amido black • Make sure HRP secondary antibody is specific for the primary antibody • “Blitzed” signal. Reduce concentration of primary and/or secondary antibody
Weak signal	• Increase film exposure time. • Place the membrane on top of a Blotting paper wetted with ROTI®Lumin during exposition • Increase conjugate concentration • Increase conjugate incubation time • Load more protein/DNA onto gel

ELISA

Excess signal or high background	• Decrease HRP conjugate concentration • Reduce conjugate incubation times • Increase blocking times • Increase washing times • Decrease the amount of protein coated to plate
No signal	• Verify the luminometer is working correctly • Make sure HRP secondary antibody is specific for the primary antibody
Weak signal	• Increase conjugate incubation time • Increase conjugate concentration • Increase the amount of protein coated to the plate

IX. Storage

Always store ROTI®Lumin stock solutions at approx. 4 °C (2-8 °C). Protect from light.
Mixed Working solution may be stored at room temperature for approx. 1 h or at 4 °C for approx 24 hs. Working solution needs not be protected from light.

X. Shelf life

ROTI®Lumin stock solutions expire 1 year after production when stored according to instructions.

XI. Content

One MINI-Kit contains:
25 ml ROTI®Lumin sol. 1 (P079)
25 ml ROTI®Lumin sol. 2 (P080)
Sufficient for 500 cm^2 membrane.

One Kit contains:
120 ml ROTI®Lumin sol. 1 (P079)
120 ml ROTI®Lumin sol. 2 (P080)
Sufficient for 2.400 cm^2 membrane.

Contents of this Kit may not be bought separately.

ROTI®Lumin	**P078.2**	1 Mini-Kit
	P078.1	1 Kit