Instructions for use

ROTI®Lumin ultra

Superior Chemiluminescent Spray-substrate for Horseradish Peroxidase (HRP)-mediated Western-Blot detections

- Very easy to use
- Spray-and-go
- Superior performance for detections in lower femtogram range
- Perfectly suited for all exposition times
- Suitable for NC membranes, optimised for PVDF membranes

I. Description and Applications

New, significantly enhanced Western-Blot substrate based on luminol, for chemoluminescent detection of horseradish peroxidase (HRP) on membranes. Through a special, optimised composition, a particularly high signal strength is achieved for over 30 mins. with a second increase at 10 to 15 mins. after application. ROTI®Lumin ultra is prepared from highly pured reagents, and has been modified by enhancers and stabilizing reagents for significantly increased sensitivity.

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ip 07/2021

ROTI®Lumin ultra is, therefore, one of the most sensitive HRP-Chemoluminescence substrate available.
By using pump spray bottles no time-consuming pre-mixing of solutions is required - substrate solution 1 and enhancer solution 2 are simply sprayed onto the membranes. Hereby, the spray technique guarantees a very smooth and regular distribution of the substrate on the membrane surface, hence avoiding artefacts caused by uneven distribution of pipetted substrate. The superior signal strength allows detection of proteins in single femtograms, as well as significant reduction of concentrations of expensive antibodies.
The use of ROTI®Lumin ultra allows for multiple stripping and reprobing of blots. ROTI®Lumin ultra is recommended for use in applications such as Western blotting, Southern blotting, dot blotting und colony hybridizations.

II. Principle

In the presence of hydrogen peroxide, HRP converts luminol to an excited intermediate dianion. This excited dianion emits light on return to its ground state.

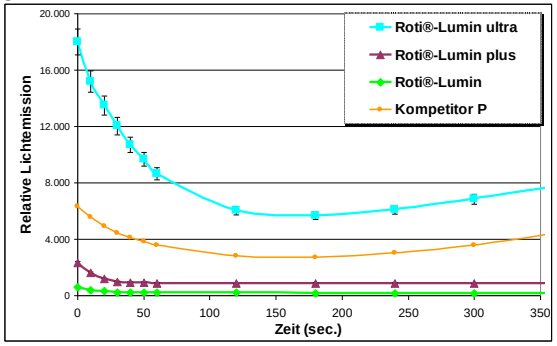

Fig. 1: Typical course of light emission of the ROTI®Lumin Group: 0 – 5 mins. after mixing/spraying.

Immediately after mixing/spraying, the light emission rate of ROTI®Lumin ultra is particularly high, staying on a very high level for several hours (see fig. 1, 2).

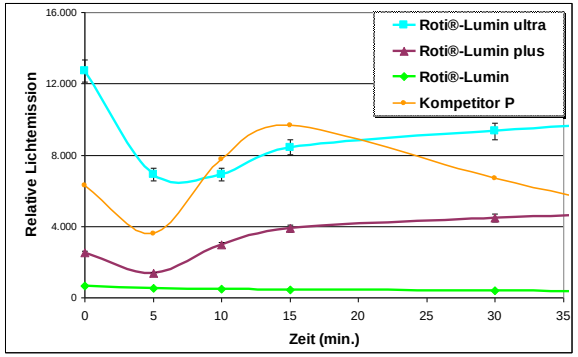

Fig. 2: Typical course of light emission of the ROTI®Lumin Group: 0 – 30 mins. after mixing/spraying.

III. Membranes

ROTI®Lumin ultra can be used with nitrocellulose (NC) and PVDF membranes. ROTI®PVDF (T830.1) is recommended for use with ROTI®Lumin ultra.

IV. Bocking reagents

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

V. Application

- Perform standard blotting and/or immunoassay procedures.
- Following incubation with HRP antibody or HRP streptavidin, perform at least three washes.
- Place the membrane onto a plastic foil (saran wrap etc.)
- Spray the membrane with solution 1. For a mini blot of 7 x 8 cm size 3-4 pump strokes are necessary.
- Immediately afterwards spray membrane with solution 2. (Same amount of pump strokes.)

- Incubate approx. 1 min. at RT, letting the solutions mix and react. During this time, wrap membrane in plastic foil and transfer membrane, film and cassette to dark room.
- Expose membrane to X-ray film for 10 secs. to 30 mins. or scan membrane in a luminometer. First exposure should be done for approx. 10-20 secs.
- Develop film.

VI. Repeated use of a blot: Removal of bound antibodies

- 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.

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)
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

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 ultra during exposition • Increase conjugate concentration • Increase conjugate incubation time • Load more protein/DNA onto gel
Precipitate visible in solution 2	• Insignificant! Does not deminish product quality. Dry milk is added for stabilising purposes (1 mg/ml), which precipitates slightly under cooling. Shake bottle slightly for resolubilisation or simply use supernatant. **Do not heat!**

IX. Storage

Always store ROTI®Lumin ultra stock solutions at approx. 4 °C (2-8 °C). Protect from light.

X. Shelf life

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

XI. Content

One MINI-Kit contains:
20 ml ROTI®Lumin ultra sol.1 in Spray-Bottle (3990)
20 ml ROTI®Lumin ultra sol.2 in Spray-Bottle (3991)
Sufficient for 2.000 cm^2 membrane (ca. 30 Mini-Gel Blots).

One Kit contains:
100 ml ROTI®Lumin ultra sol.1 in Spray-Bottle (3990)
100 ml ROTI®Lumin ultra sol.2 in Spray-Bottle (3991)
Sufficient for 10.000 cm^2 membrane (ca. 150 Mini-Gel Blots).

Contents of this Kit may not be bought separately.

ROTI®Lumin ultra

3734.1	Spray	1 Mini-Kit