For life science research only.
Not for use in diagnostic procedures.

Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)

Version 10

Content version: March 2019

Western blotting kit for the chemiluminescent detection of antigens blotted on membranes using primary antibodies from mouse or rabbit

Cat. No. 12 015 218 001

For 1,000 cm^2 of membrane surface area

Store the kit at +2 to +8°C

sigma-aldrich.com

Table of Contents

1. What this Product Does

Caution

Lumi-Light^{PLUS} Luminol/Enhancer Solution and Lumi-Light^{PLUS} Stable Peroxide Solution have not been tested to determine their hazards.

Emergency and First Aid Procedures:

Contact with eye or skin: Flush with water (at least 15 minutes for eyes) and remove contaminated clothing.

Ingestion: Seek immediate medical attention.

Kit Contents

Bottle/ Cap	Label	Contents Including function
1 (white)	Anti-Rabbit IgG-POD	• 20 U • polyclonal antibody from sheep conjugated with horseradish peroxidase (POD) • white lyophilizate • detection antibody
2 (red)	Anti-Mouse Ig-POD, Fab fragments	• 20 U • polyclonal antibody from sheep, Fab-fragment conjugated with horseradish peroxidase (POD) • white lyophilizate • detection antibody
3 (colorless)	Blocking Reagent	• 50 g • yellowish powder • Blocking of membrane and dilution of antibodies
4 (colorless)	10 × TBST (TBS-Tween 20)	• 2 × 500 ml • clear solution, foaming possible • for washing and preparation of Western Blocking Solution
5 (black)	Lumi-Light^{PLUS} Luminol/Enhancer Solution	• 50 ml • clear solution • Component of substrate solution
6 (colorless)	Lumi-Light^{PLUS} Stable Peroxide Solution	• 50 ml • clear solution • Component of substrate solution

Additional Equipment and Reagents Required

- Lumi-Imager or X-ray film cassette and X-ray film (*e.g.,* Lumi-Film Chemiluminescent Detection Film)
- Transparent films
- Reciprocal shaker
- PVDF membranes or Nitrocellulose membranes
- Blunt-ended forceps with non serrated tips
- Antigen specific antibody developed in mouse or rabbit
- Methanol, isopropanol or ethanol for wetting PVDF membranes
- Double distilled water for reconstitution and dilution purposes

Product Description

Lumi-Light^{PLUS} Western Blotting Substrate represents a new generation of chemiluminescent POD- substrates for western blotting applications.
The long lasting luminescence (>12 h) and high sensitivity (>1 pg of protein can be detected) provides enormous advantages over other conventional chemiluminescent western substrates.
Lumi-Light^{PLUS} consists of Luminol with a special enhancer and a stable peroxide solution.
It is suited for high sensitivity western blotting, especially when quantification is required.

Detection Principle

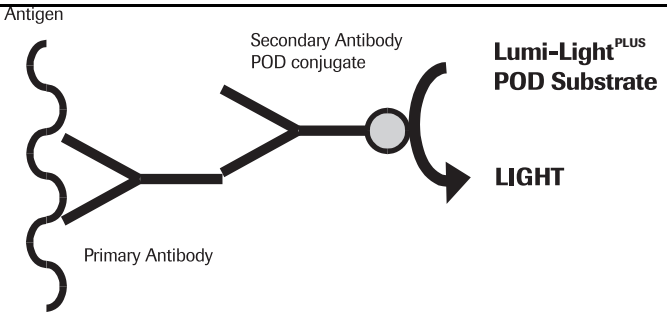

Fig. 1: Chemiluminescent detection of blotted antigens using primary and secondary antibodies and Lumi-Light^{PLUS} substrate.

Basic Steps

The following table and Figure 1 show the basic steps of the detection principle.

❶	Antigen is blotted onto the membrane.
❷	Primary antibody binds to immobilized antigen.
❸	A secondary anti-mouse/rabbit-antibody-POD conjugate binds to primary antibody.
❹	Horseradish peroxidase converts Lumi-Light^{PLUS} substrate resulting in light emission.
❺	Light is detected by exposition to X-ray film or Lumi-Imager.

Emission Wavelength

The emission wavelength of Lumi-Light^{PLUS} is 425 nm.

Time Response Curve for Lumi-Light^{PLUS} Signal

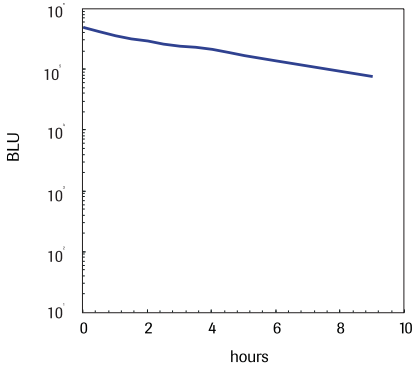

Fig. 2: Lumi-Light^{PLUS} signal in relative light units from a typical Western blot, analyzed on the Lumi-Imager Workstation.

Binding Characteristics of Anti- Mouse/ Rabbit POD Conjugates

The following table shows the binding characteristics of the secondary mouse POD conjugates.

Reactivity	Weak reactivity	No cross reactivity	Cross reactivity
with all mouse Ig classes and subclasses	<1% with Ig from horse	<0.01% with Ig from man, pig, cow, goat, mouse, or fetal bovine serum	<5% with Ig from rat and guinea pig

The following table shows the binding characteristics of the secondary rabbit POD conjugates.

Reactivity	Weak reactivity	No cross reactivity	Cross reactivity
with rabbit IgG	<1% with serum from guinea pig	<0.01% with Ig from man, cow, goat, rabbit, or horse	<10% with rat serum

Application

Detection of any antigen, blotted on PVDF or nitrocellulose membrane.

Sample Material

Any antigen which can be immobilized on PVDF or nitrocellulose membranes.

Assay Time

3 hours.

Number of Tests

The reagent is sufficient for 10 blots (conventional tank incubation) or up to 100 blots (transparency technique) with 10 × 10 cm² size each.

Quality Control Each lot is function tested using mouse and rabbit primary antibodies.

Storage/Stability The unopened kit is stable at +2 to +8°C until the expiration date printed on the label.

Sensitivity Depending on the affinity of the primary antibody 1 – 5 pg amounts of antigen can be detected.

Advantage The following table shows the benefits and features of the product.

Benefits	Features
Detection of lowest amounts of blotted proteins	Lumi-Light^{PLUS} substrate detects antigen in the range of 1 – 5 pg.
Multiple exposures possible	The signal is stable for approx. 12 hours after substrate addition.
Save primary antibody	Due to the high signal of Lumi-Light^{PLUS} substrate, the primary antibody can be diluted up to 10 – 100 fold more than with colorimetric detection systems.
Easy preparation of substrate solution	Just mix the two Lumi-Light^{PLUS} components in a 1:1 stoichiometry.

2. How to Use this Product

2.1 Before You Begin

Additional Reagents Required

- PVDF membrane or nitrocellulose membrane
- Primary antibody, antigen specific
- Alternative Blocking solution: skim milk powder or BSA
- Double distilled water for reconstitution and dilution purposes
- Methanol, isopropanol or ethanol for wetting PVDF membranes

Preparation of Additional Reagents and Solutions

The table describes the preparation of working solutions.

Volumes are designed for a membrane of 10 cm × 10 cm, if larger membranes are used, volumes must be scaled up.

For reproducible results equilibrate all solutions to room temperature before use.

⚠ Do not use azide to stabilize the solutions against microbial growth, as azide irreversibly inhibits horseradish peroxidase.

Solution	Preparation of working solution	Storage and stability	Use
Primary antibody	• Centrifuge primary antibody for 2 min at 5,000–10,000 × *g*. • Dilute an aliquot of the supernatant in 10 ml of 1% Blocking Solution. 🔍 The optimal antibody concentration should be evaluated before (see section 3.3)	1 day at +2 to +8°C	Detection protocol step 2

Preparation of Kit Working Solutions

Please refer to the following table.

Solution	Reconstitution/Preparation of working solution	Storage and stability	Use
Lumi-Light^{PLUS} substrate solution	Depending on the size of the membrane mix equal amounts of Lumi-Light^{PLUS} Enhancer (bottle 5) and Lumi-Light^{PLUS} Stable Peroxid solution (bottle 6). Mix 50 µl of each solution per cm² blot surface (conventional tank incubation) or 5 µl of each solution per cm² blot surface (transparency technique).	24 hours at +15 to +25°C	Detection protocol step 6

Solution	Reconstitution/Preparation of working solution	Storage and stability	Use
1× TBST	Dilute 100 ml 10 × TBST (bottle 4) with 900 ml double dist. water to yield 1 l of 1 × TBST solution. If you want to prepare additional 1 × TBST [50 mM Tris, 150 mM NaCl, 0.05% Tween 20 (v/v)]: • Dissolve 7.9 g Tris/HCl and 8.8 g NaCl in 900 ml double dist. water. • Adjust pH to 7.5 with 2 N NaOH. • Add 500 µl of Tween 20. • Add double dist. water to 1 l total solution.	4 weeks at +2 to +8°C	Blocking and Washing solutions
1% Blocking Solution	Prepare 1× Blocking solution from Blocking reagent (bottle 3) by dissolving 1 g in 100 ml TBST under constant stirring for approx. 30 min. Do not filter! The solution remains turbid.	1 week at +2 to +8°C	
Alternative Blocking solutions:	**BSA**: • Dissolve 5 g of BSA in 100 ml of 1× TBST with stirring. • Filter through a 0.45 µm filter. Higher concentrations of BSA may be necessary for certain antibodies.	1 week at +2 to +8°C	Antibody dilution and blocking of membrane
	Skim milk: Dissolve 2 g skim milk powder in 200 ml of TBST with stirring for at least 30 min.	1 week at +2 to +8°C	
Anti-Mouse IgG-POD	• Dissolve Anti-Mouse IgG-POD (bottle 2) in 1 ml of double distilled water. • Centrifuge the Anti-Mouse IgG-POD solution for 2 min at 5,000 - 10,000 × *g* to pellet possible aggregates. • Dilute 25 µl of Anti-Mouse IgG-POD in 10 ml of 1% Blocking Solution [50 mU/ml].	3 months at +2 to +8°C 1 day at +2 to +8°C	Detection protocol step 5

Solution	Reconstitution/Preparation of working solution	Storage and stability	Use
Anti-Rabbit IgG-POD	• Dissolve Anti-Rabbit IgG-POD (bottle 1) in 1 ml of double distilled water. • Centrifuge the Anti-Rabbit IgG-POD solution for 2 min at 5,000 - 10,000 × *g* to pellet possible aggregates. • Dilute 10 µl of Anti-Rabbit IgG-POD in 10 ml of 1% Blocking Solution [20 mU/ml].	3 months at +2 to +8°C 1 day +2 to +8°C	Detection protocol step 5

2.2 Preparation of Membrane

Electrophoresis and Electrotransfer

- To carry out electrophoresis either use non-denaturing gels, SDS-PAGE or two-dimensional gels according to common protocols (2, 3).
- Perform electrotransfer according to common protocols (4, 5).
- After transfer, blotting efficiency can be checked by reversibly staining the transferred proteins with Ponceau S solution.

General Handling Recommendations

The table describes general hints for the preparation of membranes.

Recommendation	Guideline
Membrane handling requirements	• Handle membrane only at the edges and with clean blunt-ended forceps. • Clean scissors before cutting the membrane with an ethanol moistened towel. • Wear powder free gloves. • Make sure sufficient solution is present to cover the membrane entirely.
Washing requirements	• Use large volumes of washing solution for all washing steps; at least 0.4 ml/cm^2 of membrane area are recommended. • Rinse briefly with washing solution prior to the washing steps to further increase the efficiency.
Special handling of PVDF membrane	• Wet hydrophobic PVDF membranes by a short rinse in methanol, isopropanol or ethanol, whereby the membrane changes color from white to grey translucent. Then wet the membrane in transfer buffer for 3 min. Note: Do not use the membrane, if parts of the membrane remain white. • PVDF membranes must not dry out at any step. If drying occurs re-wet in 5% Tween® 20 (v/v). This may, however, influence antibody binding.

Storage of Membrane after Blotting

This table describes how you must store the membrane after blotting.

IF...	THEN...
You want to stop	Store dry membrane at +2 to +8°C (up to 3 months). After storage start with Detection protocol step 1a.
You want to go ahead	Start with Detection protocol with step 1b.

2.3 Optimization of Antibody Concentrations

General Introduction

Since Lumi-Light^{PLUS} gives very strong signals, compared to conventional POD substrates, we highly recommend optimizing the antibody concentration in your experimental system.

First optimize the concentration of the primary antibody using a constant amount of secondary antibody-POD conjugate (*e.g.,* 50 mU/ml). Then, using the optimized primary antibody concentration, adjust the concentration of secondary antibody-POD conjugate.

Optimization of Primary Antibody Concentrations

The following procedure can be used to optimize antibody concentrations for the Lumi-Light^{PLUS} substrate solution.

❶	• Cut a piece of nitrocellulose or PVDF membrane for each antibody concentration to be tested. • Spot an appropriate amount of antigen onto individual pieces of membrane. Air dry the samples if nitrocellulose membranes are used.
❷	Block the membranes in 1% Blocking Solution (0.4 ml/cm²) for 1 h under constant shaking.
❸	• Prepare primary antibody dilutions with 1% Blocking Solution, *e.g.,* 1:100, 1:300, 1:1,000, 1:3,000, 1:10,000 • Incubate one piece of membrane in each dilution for 1 h under constant shaking.
❹	First rinse and then wash membranes 4 × 2 min with 1 × TBST (0.4 ml/cm²).
❺	Depending on which primary antibody was used add secondary antibody-POD (*e.g.,* Anti-Rabbit IgG-POD at a concentration of 20 mU/ml or Anti-Mouse IgG-POD at a concentration of 50 mU/ml) in 1% Western Blocking Solution (0.1 ml/cm²) and incubate membranes 30 min under constant shaking.
❻	• Rinse membranes, 4 × 2 min with 40 ml of TBST. • Drain TBST from the membranes by holding it with forceps and briefly blotting the lower edge onto absorbent filter paper.

❼	• Incubate for 5 min with 0.1 ml/cm² Lumi-Light^{PLUS} substrate solution in a glass tray or disposable petri dish. The membranes must be soaked entirely with substrate solution and must not dry out during this step. • Remove the membrane from the Lumi-Light^{PLUS} substrate solution and place it protein side up on transparency film. • Cover the membranes with a second transparency film and remove air bubbles.
❽	• Gently squeeze out excess liquid onto an adsorbent filter paper. • Seal the membranes.
❾	• Expose on Lumi-Imager or X-ray film for 1 min. • Place another sheet of X-ray film on the membrane, while developing the first film. • Choose the most suitable dilution of antibodies yielding the highest signal and minimal background.

Optimization of Secondary Antibody Concentrations

The following table describes the optimization of the secondary antibody.

❶	• Cut a piece of nitrocellulose or PVDF membrane for each antibody concentration to be tested. • Spot an appropriate amount of antigen onto individual pieces of membrane. Air dry the samples if nitrocellulose membranes are used.
❷	Block the membranes in 1% Blocking Solution (0.4 ml/cm²) for 1 h under constant shaking.
❸	Incubate the membranes in the optimized primary antibody concentration from the previous protocol for 1 h under constant shaking.
❹	Rinse membranes 4 × 2 min with 1 × TBST (0.4 ml/cm²).
❺	• Prepare suggested secondary antibody dilutions with 1% Western Blocking Solution *e.g.,* 200 mU/ml, 100 mU/ml, 50 mU/ml, 25 mU/ml, 10 mU/ml and 5 mU/ml. • Incubate one piece of membrane in each dilution for 1 h under constant shaking. For antibodies of other origin, other dilutions (1:1,000–1:50,000) may be necessary.
❻	• Rinse membranes, 4 × 2 min with TBST (0.4 ml/cm²). • Drain TBST from the membranes by holding it with forceps and briefly blotting the lower edge onto absorbent filter paper.

❼	• Incubate for 5 min with (0.1 ml/cm²) Lumi-Light^{PLUS} substrate solution in a glass tray or disposable petri dish. The membranes must be soaked entirely with substrate solution and must not dry out during this step. • Remove the membrane from the Lumi-Light^{PLUS} substrate solution, place it protein side up on transparency film and cover the membranes with a second transparency film.
❽	• Gently squeeze out excess liquid and air bubbles onto an adsorbent filter paper. • Seal the membranes.
❾	• Expose on Lumi-Imager or X-ray film for 1 min. • Then place another sheet of X-ray film on the membrane, while developing the first film. • Choose the most suitable dilution of antibodies yielding the highest signal and minimal background.

2.4 Detection protocol

Procedure

This procedure is designed for a membrane of 10 cm × 10 cm, if larger membranes are used volumes must be scaled up.

All steps are performed at +15 to +25°C and with gentle agitation on a reciprocal shaker.

❶ a optional

IF...	THEN...
NC membrane was stored at +2 to +8°C.	Wet NC membranes by a short rinse in water
PVDF membrane was stored at +2 to +8°C.	Wet PVDF membrane by a short rinse in methanol, isopropanol or ethanol then wash with double dist. water before proceeding to the next step.
Blotting was performed in a buffer system containing methanol	Briefly wash the membrane 3 × 2 min with 40 ml TBST (0.4 ml/cm²) to avoid background staining.

❶ b Add 40 ml 1% Blocking Solution, to an appropriate incubation tray and incubate the membrane under constant shaking for 1 h or overnight at +2 to +8°C without shaking.

If blotting was performed in a buffer containing methanol, briefly wash the membrane 3 × 2 min with 40 ml TBST (0.4 ml/cm²) to avoid background staining.

❷ Incubate membrane with 10 ml of primary antibody solution under constant shaking for 1 h or overnight at +2 to +8°C without shaking.

Extend incubation time to overnight, if either the affinity of the antibody to the antigen, or if the concentration of specific antibody is low.

❸ First rinse and then wash 4 × 2 min with 40 ml of TBST.

❹ Add 10 ml of appropriate secondary antibody-POD solution and incubate membrane 30 min under constant shaking.

❺
- First rinse and then wash 4 × 2 min with 40 ml of TBST.
- Drain TBST from the membrane by holding it with forceps and briefly blotting the lower edge onto absorbent filter paper.

❻

Conventional tank blot	• Incubate for 5 min with 10 ml Lumi-Light^{PLUS} substrate solution in a glass tray or disposable petri dish. The blot must be soaked entirely with substrate solution and must not dry out during this step. • Remove the membrane from the Lumi-Light^{PLUS} substrate solution and place it protein side up on transparency film. • Cover the membrane with a second transparency film and remove air bubbles. Ⓢ For increased sensitivity the substrate incubation time can be increased to up to 30 min.
Transparency technique	• Place the membrane protein side up on a sheet of transparency film. • Add 1 ml Lumi-Light^{PLUS} substrate solution onto the membrane. • Immediately cover the membrane with a second transparency film, remove air bubbles and incubate for 5 min. Ⓢ For increased sensitivity the substrate incubation time can be increased up to 30 min.

❼
- Gently squeeze out excess liquid onto an adsorbent filter paper.
- Seal the membrane.

❽
- Expose on Lumi-Imager or X-ray film for 1 min.
- Then place another sheet of X-ray film on the membrane, while developing the first film.
- Adjust the exposure time between 10 sec and up to 1 h according to the result with the first film.

Ⓢ The X-ray film must not become wet.

Ⓢ For comparison of blots it is necessary to use the same substrate incubation time, since the signal intensity may increase. The luminescent reaction reaches its maximum after approx. 10 min.

2.5 Stripping and Reprobing of Blots

Additionally Required Solutions

- TBST [50 mM Tris, 150 mM NaCl, 0,05% Tween 20 (v/v)]
- Stripping solution [62.5 mM Tris-HCl, pH 6.8 (1 g), 2% SDS (2 g), 100 mM β-mercaptoethanol]

Procedure

The following procedure describes the stripping and reprobing of blots.

❶	Incubate blot for 30 min at +70°C in stripping solution.
❷	Wash 3 × 5 min with TBST.
❸	Go ahead with the Detection protocol (section 3.4).

3. Troubleshooting

Problem	Possible Cause	Recommendation
No or weak signal	Inefficient protein transfer	• Check protein transfer efficiency with Ponceau S solution or Silver staining of the gel after blotting. • Change transfer conditions if efficiency is low.
	Primary antibody does not detect denatured (in denaturing gels containing SDS or Urea) proteins on blots.	Perform a dot blot with denatured protein and native protein in parallel. If the primary antibody only binds to native protein, try to use non-denaturing gel systems.
	Affinity of primary antibody is low	• Optimize antibody concentration according to section 3.3. • Prolong incubation with primary antibody to overnight at +2 to +8°C. • Shorten washing times and use washing buffer without Tween 20. • Incubate primary and secondary antibodies in buffer without Western Blocking Reagent (background may be increased).
	POD-activity of the secondary antibody has decreased	Dot blot different dilutions of POD-conjugate onto a blotting membrane and detect directly according to section 3.3 1. If no signal appears, use fresh POD-conjugate and test in the same way. 2. If still no signal appears, use new Lumi-Light^{PLUS} substrate solution.
	Detection reagent gives no signal	• Check if Lumi-Light^{PLUS} substrate solution was equilibrated +15 to +25°C. • Use non-expired, non-contaminated Lumi-Light^{PLUS} substrate solution.
	Concentrations of secondary antibody to low	• Prolong incubation time with secondary antibody to 3 h. • Prolong detection time.
	Insufficient amount of protein loaded	Increase amount of protein applied onto the gel.
Background problems	Inadequate washing	Prolong washing times, increase number of washes.
	Inadequate blocking	• Block overnight. • Increase concentration of Western Blocking Solution up to 10%. • Use fresh Western Blocking Solution.

Problem	Possible Cause	Recommendation
Spotted or uneven background	Concentration of primary and secondary antibodies too high	As Lumi-Light^{PLUS} gives very high signals, it is absolutely essential to optimize antibody concentrations (see section 3.3). In general, antibody concentrations must be diluted tenfold in comparison to conventional chemiluminescence substrates.
	Overexposure of film	Use shorter exposition time.
	Membranes dried partially during the procedure	Avoid drying of the membrane at any time during the procedure.
	Membranes were not submerged entirely during blocking or washing steps	Carefully check that the membrane is always covered with buffer during the incubations and moves freely in the working solutions.
	Primary antibody and/or POD conjugate aggregates	Always centrifuge the primary antibody and POD conjugate and use supernatant before diluting in Western Blocking Solution. Alternatively, filter through a 0.2 µm filter with low protein adsorption.
	Contamination of equipment or solutions	• Use clean equipment. • Prepare fresh buffers.
	Contamination of membranes	• Use new membranes. • Follow general handling instructions of membranes in section 3.2.

4. Additional Information on this Product

4.1 References

1 Mattson, D.L. & Bellehumeur T.G. (1996) Comparison of three chemiluminescent horseradish peroxidase substrates for immunoblotting. *Anal. Biochem*. **240**, 306.

2 Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4 *Nature* **227**, 680.

3 Hames, B.D. & Rickwood, D. eds. (1981) Gel electrophoresis of proteins: A practical approach. IRL press, Oxford.

4 Burnette, W.N. (1981) "Western Blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem*. **112**, 195.

5 Towbin, H.T. *et al* (1979) Electrophoretic transfer of proteins from acrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci*. **76**, 4350.

6 Tesfaigzi, J.*et al* (1994) A simple method for reusing western blots on PVDF membranes. *BioTechniques* **17**, 268.

7 Kaufmann, S.H. & Shaper, J.H. (1992) Erasable Western Blots. in Methods in Molecular Biology Vol. 10: Immunochemical Protocols M. Manson eds. Humana Press Inc. pp 235-246.

5. Supplementary Information

5.1 Conventions

Text Conventions To make information consistent and easy-to-read, the following text conventions are used in this document:

Text Convention	Usage
Numbered stages labeled ①, ② *etc.*	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷ *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics..

Symbols In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

- Editorial changes.

5.3 Ordering Information

Kits

Product	Pack Size	Cat. No.
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	Reagents for 2000 cm^2 of membrane surface area	11 520 709 001

Single reagents

Product	Pack Size	Cat. No.
Bovine Serum Albumin	100 g	10 735 086 001
Lumi-Film Chemiluminescent Detection Film	100 films (18 × 24 cm)	11 666 916 001
Lumi-Light Western Blotting Substrate	100 ml	12 015 200 001

Product	Pack Size	Cat. No.
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml	12 015 196 001
Nylon membranes, positively charged	20 sheets (20 × 30 cm) 10 sheets (10 × 15 cm) 1 roll (0.3 × 3 m)	11 209 299 001 11 209 272 001 11 417 240 001
PVDF Western blotting membranes	10 sheets 15 × 15 cm each 1 roll 26.5 cm × 3.75 m	11 722 026 001 11 722 034 001
Tris-HCl	500 g	10 812 846 001
Tween 20	5 × 10 ml	11 332 465 001
Western Blotting Reagent	100 ml 6 × 100 ml	11 921 673 001 11 921 681 001

5.4 Trademarks

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5.5 Regulatory Disclaimer

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Sandhofer Strasse 116
68305 Mannheim
Germany