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

BM Chemiluminescence Western Blotting Substrate (POD)

Version: 09
Content Version: December 2020

For detection of proteins by western blotting and nucleic acids.

Cat. No. 11 500 708 001	1 set 1,000 cm^2 membrane (trays), 6,250 cm^2 membrane (transparent plastic bags)
Cat. No. 11 500 694 001	1 set 4,000 cm^2 membrane (trays), 25,000 cm^2 membrane (transparent plastic bags)

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	BM Chemiluminescence Blotting Substrate (POD), Luminescence substrate solution A	Detection reagent	11 500 708 001	1 bottle, 125 ml
			11 500 694 001	2 bottles, 250 ml each
2	BM Chemiluminescence Blotting Substrate (POD), Starting solution B	To prepare Detection reagent.	11 500 708 001	1 bottle, 2 ml
			11 500 694 001	3 bottles, 2 ml each
3	BM Chemiluminescence Blotting Substrate (POD), Blocking reagent	▪ 10% (w/v) stock solution ▪ For blocking of nonspecific binding sites.	11 500 708 001	1 bottle, 100 ml
			11 500 694 001	3 bottles, 100 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Luminescence substrate solution A	Store at +2 to +8°C.
2	Starting solution B	
3	Blocking reagent	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Standard electrophoresis apparatus
- Centrifuge
- Powder-free gloves
- Saran wrap
- Transparency film
- Reciprocal shaker or roller incubator
- Filter paper
- Blunt-ended forceps with non serrated tips
- Incubation trays or transparent plastic bags

  ⚠ ***The volumes for the washing and incubation solutions recommended in the procedure are only applicable when the size of the incubation trays fits the size of the membrane. Only use disposable trays, or trays, which are carefully cleaned.***
- X-ray film

For preparation of solutions

See section, **Working Solution** *for information on preparing solutions.*

- TBS (Tris buffered saline)
- TBST (TBS-Tween 20)
- Tris base*
- Tween 20*
- Water, PCR Grade*

For detection of proteins

- PVDF Western Blotting Membranes*, or
- Nitrocellulose membranes
- Primary antibody, (peroxidase-labeled)
- Secondary reagents (antibody, streptavidin)
- Methanol (wetting the PVDF membranes)

For hybridization of nucleic acids

See section, **Working Solution** *for information on preparing solutions.*

- Standard buffer
- Standard buffer + 50% formamide
- High SDS buffer
- High SDS buffer + 50% formamide

For detection of nucleic acids

See section, **Working Solution** *for information on preparing solutions.*

- 10% N-laurylsarcosine (w/v)
- 10% SDS* (w/v)
- 20x SSC*
- Maleic acid solution
- Maleic acid solution + 0.3% Tween 20 (v/v)
- 1% Blocking solution (w/v)
- 0.5% Blocking solution (w/v)
- Detection solution

For stripping and reprobing of protein blots

- 2-mercaptoethanol
- 2% SDS*

For stripping and reprobing of nucleic acid blots

- 50 mM EDTA
- 0.1% SDS* or 1% SDS*
- 2x SSC
- 0.2 M NaOH
- 50 mM Tris-HCl*
- Water, PCR Grade*
- Dimethylformamide

1.4. Application

Proteins

The BM Chemiluminescence Blotting Substrate (POD) is especially suited for:

- Western and dot blot applications where high sensitivity is required.

  Chemiluminescence detection of proteins is 1 to 3 orders of magnitude more sensitive than colorimetric methods. Compared with radioactive detection of blotted antigens, the chemiluminescence image is at least as sensitive after much shorter exposure times and without the risk of working with radioactivity.

Nucleic acids

The sensitivity and specificity of the BM Chemiluminescence Blotting Substrate (POD) makes it useful for:

- All hybridization techniques where radioactive labeling is normally required.

  The detection system can be used after transfer of electrophoretically separated DNA and RNA onto nylon or nitrocellulose membranes.
- Colony and plaque screening.
- Ease of reprobing and speed of results makes it especially suited for all applications where blots must be hybridized with different multiple probes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Electrophoresis and electrotransfer

- Carry out electrophoresis using either non-denaturing gels, SDS-PAGE, or two-dimensional gels according to standard protocols.
- Blot according to standard protocols.

  The best results have been obtained using PVDF Membranes, but nitrocellulose membranes can also be used.*

Membrane handling requirements

Follow good laboratory practice when handling membranes.

- Handle membrane only on the edges and with clean blunt-ended forceps.
- Clean scissors with an ethanol moistened towel before cutting the membrane.
- Wear powder-free gloves to avoid damage or contamination.
- Make sure that there is sufficient solution to entirely cover the membrane.

Special handling of PVDF membranes

- Wet hydrophobic PVDF membranes with a brief rinse in methanol; isopropanol, or ethanol; the membrane changes color from white to gray translucent.
- Wet the membrane in transfer buffer for 3 minutes.

  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.

Optimal hybridization conditions for different probe types

For DNA

Type of blot	Concentration [ng/ml]	Hybridization Solution	Conditions
Southern blot	5 – 25	Standard buffer	Hybridize overnight at +68°C.
		Standard buffer + 50% Formamide	Hybridize overnight at +37 – +42°C.
		High SDS buffer	
		High SDS buffer + 50% Formamide	Hybridize overnight at +55°C.
Northern blot	5 – 25	High SDS buffer	Hybridize overnight at +50°C.
Colony and plaque	100		

For RNA

Type of blot	Concentration [ng/ml]	Hybridization Solution	Conditions
Southern blot	100	Standard buffer + 50% Formamide	Hybridize overnight at +50°C.
Northern blot	100		Hybridize overnight at +68°C.
Colony and plaque	5 – 25	Standard buffer	
		Standard buffer + 50% Formamide	Hybridize overnight at +42°C.

For oligonucleotides

For tailed or end-labeled oligonucleotides, use the following conditions.

⚠ ***Perform hybridization with a tailed oligonucleotide with 0.1 mg/ml Poly(A)* and 5 µg/ml Polyd(A)*, both in the prehybridization and hybridization solution to prevent nonspecific hybridization signals.***

Type of blot	Concentration [pmol/ml]	Hybridization Solution	Conditions
Southern blot	0.1 – 2 (tailed) 1 – 10 (end-labeled)	Standard buffer	▪ Hybridize 1 to 6 hours. ▪ Temperature varies considerably and can be approximated by considering probe length and G plus C content.
Northern blot			
Colony and plaque			

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of kit working solutions			
Solution	**Preparation/Composition**	**Storage and Stability**	**For use in...**
1% Blocking solution	Add 10 ml of Blocking reagent stock solution (w/v) (Bottle 3) to 90 ml TBS. ⚠ ***125 µl/cm² of membrane is required.***	Store at −15 to −25°C.	Membrane blocking
0.5% Blocking solution	Add 5 ml of Blocking reagent stock solution (w/v) (Bottle 3) to 95 ml TBS.		Antibody dilution
Detection solution	Mix substrate solution A (Bottle 1) and Starting solution B (Bottle 2) in a ratio of 100:1; allow to equilibrate at +15 to+25°C. ⚠ ***20 µl/cm² of membrane is required if plastic bags are used for detection; 125 µl/cm² of membrane is required if trays are used for detection.***	Store 1 week at +2 to +8°C. ⚠ ***Keep protected from light.***	Detection protocol
Preparation of solutions for proteins			
Solution	**Preparation/Composition**	**Storage and Stability**	**For use in...**
TBS (50 mM Tris, 150 mM NaCl)	▪ Dissolve 6.05 g Tris base* (50 mM) and 8.76 g NaCl (150 mM) in 800 ml PCR-grade water*. ▪ Adjust pH to 7.5 with approximately 9.5 ml 1 M HCl ▪ Dilute up to 1 l total volume with double-distilled water. ⚠ ***Do not use sodium azide as an antimicrobial agent as it inhibits POD.***	Store 3 months at +2 to +8°C.	Blocking and washing solutions.
TBST	Dilute 1 ml Tween 20* to 0.1% (v/v) final concentration in 1 l TBS.		Wash solution
Primary antibody (POD-labeled)	Dilute conjugated or unconjugated antibody in 0.5% Blocking solution. ℹ *4 µg/ml of primary antibody are usually sufficient for sensitive detection.* ⚠ ***125 µl/cm² of membrane is required.***	⚠ ***Always prepare fresh.***	Detection protocol
Secondary reagents (antibody, streptavidin)	20 mU/ml of peroxidase-labeled secondary reagent (antibody, streptavidin) is usually sufficient for sensitive detection. ⚠ ***125 µl/cm² of membrane is required.***	–	

Preparation of solutions for nucleic acids			
Solution	**Preparation/Composition**	**Storage and Stability**	**For use in...**
10% N-laurylsarcosine (w/v)	Dissolve 10 g N-laurylsarcosine in 80 ml of PCR-grade water, adjust to 100 ml with PCR-grade water, and filter through a 0.2 to 0.45 µm membrane.	Store at +15 to +25°C. ⚠ ***Keep sterile.***	Preparing hybridization solutions.
10% SDS* (w/v)	Dissolve 10 g SDS in 80 ml of PCR-grade water, adjust to 100 ml with PCR-grade water, and filter through a 0.2 to 0.45 µm membrane.		
20x SSC*	▪ Dissolve 175.32 g NaCl (3 M), 88.23 g sodium citrate (0.3 M) ($C_6H_5Na_3O_7 \times H_2O$) in 800 ml with PCR-grade water. ▪ Adjust pH to 7.0, adjust to 1 l with PCR-grade water, and autoclave.		
Maleic acid solution	▪ Dissolve 11.61 g Maleic acid (0.1 M) 8.77 g NaCl (0.15 M) in 800 ml PCR-grade water. ▪ Adjust pH to 7.5 with concentrated NaOH; adjust to 1 l with PCR-grade water, and autoclave.		Detection protocol
Maleic acid solution plus 0.3% Tween 20* (v/v)	Add 0.3 ml of Tween 20 to 100 ml of Maleic acid solution. ⚠ ***Prepare from sterile stock solution.***		
Preparation of hybridization solutions ℹ *To choose a suitable hybridization solution, see section, Optimal hybridization conditions for different probe types.*			
Solution	**Preparation/Composition**	**Storage and Stability**	**For use in...**
Standard buffer	Mix 25 ml 20x SSC (5x SSC), 10 ml 1% Blocking reagent (1%), 1 ml 10% N-laurylsarcosine (0.1%), 0.2 ml 10% SDS (0.02%), and 63.8 ml PCR-grade water.	Store at −15 to −25°C.	Optimizing hybridization conditions.
Standard buffer plus 50% Formamide*	Mix 25 ml 20x SSC (5x SSC), 20 ml Blocking reagent (2%), 1 ml 10% N-laurylsarcosine (0.1%), 0.2 ml 10% SDS (0.02%), 50 ml deionized Formamide, 3.8 ml PCR-grade water.		
High SDS buffer	▪ Dissolve 7 g SDS (7%) in 25 ml 20x SSC (5x SSC), 20 ml Blocking reagent (2%), 1 ml 10% N-laurylsarcosine, (0.1%), 5 ml 1 M sodium-phosphate (0.05 M), pH 7.0. ▪ Adjust with PCR-grade water to 100 ml.		
High SDS buffer plus 50% Formamide*	▪ Dissolve 7 g SDS (7%) in 25 ml 20x SSC (5x SSC), 2 g Blocking reagent (2%), 1 ml 10% N-laurylsarcosine (0.1%), 50 ml deionized Formamide, 5 ml 1 M sodium-phosphate (0.05 M), pH 7.0. ▪ Adjust with PCR-grade water to 100 ml.		
Washing solution, 2x conc.	Mix 890 ml double-distilled water, 100 ml 20x SSC (2x SSC), 10 ml 10% SDS (0.1%).	Store at +15 to +25°C. ⚠ ***Autoclave, keep sterile.***	Detection protocol
Washing solution, 0.1x conc.	Mix 985 ml double-distilled water, 5 ml 20x SSC (0.1x SSC), 10 ml 10% SDS (0.1%).		

2.2. Protocols

Electrophoresis, blotting, and processing of blots

Protein analysis

⚠ ***This procedure is designed for a membrane of 10 cm × 10 cm; if larger membranes are used, scale up the volumes.***

ⓘ *Perform all steps at +15 to +25°C and with gentle agitation on a reciprocal shaker or a roller incubator. The roller incubator minimizes reagent consumption since only 10 ml of solution is needed for a 10 × 10 cm membrane, resulting in even reagent distribution and avoids drying out of the membrane. When using a shaker, make sure that enough solution is present to entirely cover the membrane. For reproducible results, equilibrate all solutions to +15 to +25°C before use.*

ⓘ *See section,* **Working Solution** *for additional information on preparing solutions.*

1. Prewet hydrophobic membranes, such as PVDF prior to protein transfer.
   – Moisten the membrane with methanol for several seconds and then soak with transfer buffer for 3 minutes.
   – The blot can be stored dry for several months at +2 to +8°C but must be re-wetted before starting the immunodetection. PVDF membranes should be re-wetted in methanol or in 5% Tween 20 (v/v), solution.

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2. If blotting was performed in a buffer system containing methanol, briefly wash the membrane 2 times with TBS.

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3. Block nonspecific binding of antibody by incubating the membrane for 1 hour in 1% Blocking solution (w/v).
   – Alternatively, this step can be performed overnight at +2 to +8°C without shaking.

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4. Incubate the membrane for 1 hour with primary antibody diluted in 0.5% Blocking solution (w/v).
   ⓘ *Extend incubation time to overnight, if either the affinity of the antibody to the antigen, or if the concentration of specific antibody is low. 4 µg/ml of specific primary or polyclonal purified antibodies is usually sufficient.*
   ⚠ ***If the primary antibody is already biotin-, DIG-, or fluorescein-labeled, continue to Step 8.***

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5. Wash twice in TBST for 10 minutes each.
   – Wash twice with 0.5% Blocking solution (w/v) for 10 minutes each.
   – For efficient washing, always use large volumes of TBST, for example, 30 ml for a 10 × 10 cm membrane.

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6. For hapten-labeled secondary antibody, such as biotin, DIG, and fluorescein, incubate the membrane for 30 minutes with secondary antibody diluted in 0.5% Blocking solution (w/v).
   ⓘ *4 µg/ml of secondary antibody is usually sufficient for highly sensitive detection.*

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7. Wash 2 times with large volumes of TBST and 2 times with 0.5% Blocking solution (w/v/) for 10 minutes each.

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8. Incubate the membrane for 30 minutes with POD-labeled secondary reagent diluted with 0.5% Blocking solution (w/v).

ⓘ *20 mU/ml or less is usually sufficient for highly sensitive detection.*

⚠ ***During incubation, prepare the Detection solution, see section, Working Solution.***

– Wash 4 times with large volumes of TBST for 15 minutes each.

Nucleic acid analysis

i *See section,* **Working Solution** *for additional information on preparing solutions.*

1. Carry out electrophoresis according to standard protocols.

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2. Transfer DNA or RNA to be probed to a nylon or nitrocellulose membrane by dot blot, plaque lift, colony lift, Southern and northern transfer according to standard protocols.
   – When transfer is carried out with a neutral transfer buffer, bind the nucleic acids by baking or by UV-crosslinking.
   – For baking, place the dried membrane for 30 minutes to 2 hours at +80°C in a vacuum or a conventional oven.
   – For UV-crosslinking, expose membrane to a source of ultraviolet irradiation (254 nm); 3 minutes is usually sufficient.
   ⚠ ***Over irradiation results in the covalent attachment of a higher proportion of thymine, with a consequential decrease in hybridization signal. After alkaline transfer, UV-crosslinking is less effective than baking. The blot can be stored dry for several months in at +2 to +8°C if necessary, but must be re-wetted before starting immunodetection.***

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3. Prehybridize filter in a hybridization bottle (hybridization oven), sealed plastic bag, or tray with hybridization solution.
   – To choose the most suitable hybridization solution and hybridization temperature for Southern, northern, and colony hybridization, see section, **General Considerations**. The prehybridization solution differs from the hybridization solution only by the absence of the probe.
   ⚠ ***Do not allow the filters to dry out between prehybridization and hybridization***

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4. Replace prehybridization solution with hybridization solution containing the DIG-, biotin- or fluorescein-labeled DNA or RNA probe.
   – Denaturation of DNA probes is carried out by heating the sample (100 µl or less) for 5 minutes at +100°C and rapidly chilling in ice water.
   – The hybridization conditions and probe concentrations for Southern, northern, and colony hybridization are described in section, **General Considerations**.

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5. Wash membrane 2 times in 2x Washing solution for 5 minutes each at +15 to +25°C and 2 times in 0.1x Washing solution for 15 minutes each.
   ⚠ ***If long probes >100 bp are used, perform the last two washes at +68°C. For shorter probes, the washing temperature corresponds to the hybridization temperature, see section, General Considerations. Filters can then be used directly for detection of hybridized probe or stored air-dried for later detection.***

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6. Transfer membrane to a fresh tray and wash briefly with Maleic acid solution.
   – Block nonspecific binding of antibody by incubating the membrane for 1 hour in 1% Blocking solution (w/v).
   – Alternatively, this step can be performed overnight at +2 to +8°C without shaking.

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7. Incubate the membrane for 1 hour with POD-labeled anti-hapten antibody or streptavidin-POD diluted in 1% Blocking solution (w/v).
   i *10 mU/ml are usually sufficient for highly sensitive detection.*
   ⚠ ***During incubation, prepare the Detection solution, see section, Working Solution.***

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8. Wash 2 times with large volumes of Maleic acid solution plus 0.3% Tween 20 for 15 minutes each and once with Maleic acid solution for 15 minutes.

Chemiluminescent detection of proteins and nucleic acids

Handling instructions

After exposing the blot to the detection reagents, it must be processed very quickly to avoid fading of the chemiluminescence reaction.

⚠ ***Always work in a dark room and have the following materials nearby.***

- X-ray film and cassette
- Timer, developer, water bath, and fixing solution in tanks
- Transparent plastic bag or tray and Saran Wrap
- Filter paper
- 10 ml glass pipette (only for development in transparent plastic bag)

Use of trays for development

1. Drain the excess buffer from the washed blot and place it, sample side up, in a fresh tray, about the same size as the blot.

2. Add premixed Detection solution.
   ℹ *Approximately 125 µl/cm² of solution is sufficient to cover the membrane.*
   – Incubate for 60 seconds.
   – Drain off excess Detection solution and wrap the blot in Saran Wrap; make sure that no air bubbles are trapped, or place the membrane between two transparent films, the type commonly used for overhead projection.

3. Insert the membrane, protein or nucleic acid side up, into a film cassette.

4. Turn off the light, place a sheet of film onto the blot, and close the cassette.
   – Expose for 10 to 60 seconds.

5. Immediately replace the exposed film with a new one, reclose the cassette, and immediately develop the exposed film.
   ⚠ ***The developing process can be followed by using a red safety light.***

6. Expose the second film for a suitable time (up to 1 hour), estimated from the signal intensity on the first film.

ℹ *The luminescent reaction reaches its maximum after 1 to 2 minutes and is relatively constant for 20 to 30 minutes. After 1 hour, the signal intensity decreases to about 60 to 70% of maximum. If signal intensity was too high, wait for 10 minutes before re-exposing the film.*

Use of transparent plastic bags for development

1. Use a transparent plastic bag about the same size as the blot (prepare the required size using a sealing device). Fill the bag with Washing buffer to prevent drying of the membrane.
   – Transfer the membrane to the bag.
   – Place the open bag onto sheets of filter paper and press off excess fluid by rolling a 10 ml glass pipette over the bag towards the open end; avoid pressing too hard.

2. Add premixed Detection solution.
   ℹ *Approximately 20 µl/cm² of solution is sufficient for high sensitivity detection.*
   – Immediately seal the bag after eliminating any air bubbles, and distribute the fluid over the entire filter surface using a 10 ml pipette.

3. Incubate for 60 seconds.

4. Insert the membrane, protein or nucleic acid side up, into a film cassette.

5. Turn off the light, place a sheet of film onto the blot, and close the cassette.
   – Expose for 10 to 60 seconds.

6. Immediately replace the exposed film with a new one, reclose the cassette, and immediately develop the exposed film.
   ⚠ ***The developing process can be followed by using a red safety light.***

7. Expose the second film for a suitable time (up to 1 hour), estimated from the signal intensity on the first film.

The luminescent reaction reaches its maximum after 1 to 2 minutes and is relatively constant for 20 to 30 minutes. After 1 hour, the signal intensity decreases to about 60 to 70% of maximum. If signal intensity was too high, wait for 10 minutes before re-exposing the film.

Stripping and reprobing of protein blots

Western blots

1. Incubate membrane in TBS containing 100 mM 2-mercaptoethanol and 2% SDS with gentle shaking for 30 minutes at +50°C.
2. Wash the membrane 2 times for 15 minutes each in a large volume of TBST at +15 to +25°C.
3. Block the membrane in 1% Blocking solution (in TBS) for 1 hour at +15 to +25°C; proceed as described in the **Detection protocol**.

Stripping and reprobing of nucleic acid blots

Southern and dot hybridizations

1. Wash the membranes twice for 15 minutes each in 50 mM EDTA, pH 8.0, 2x SSC at +85°C.
2. Wash 2 times for 5 minutes each in 2x SSC, 0.1% SDS.
3. Wash 2 times for 15 minutes each in 0.1% SDS, 0.2 M NaOH at +37°C.
4. Rinse the membrane briefly in 2x SSC.
5. Continue reprobing with the prehybridization step of the desired hybridization protocol.

Northern hybridization

1. Rinse the membrane thoroughly in PCR-grade water.
2. Incubate the membrane 2 times for 30 minutes each in 50% dimethylformamide, 1% SDS; 50 mM Tris-HCl, pH 8.0 at +68°C.
3. Rinse the membrane first in water then in 2x SSC.
4. Continue reprobing with the prehybridization step of the desired hybridization protocol.

3. Troubleshooting

For protein analysis

Observation	Possible cause	Recommendation
No or weak signal present.	Inefficient protein transfer.	Check protein transfer efficiency by silver staining of the gel after blotting.
		Change transfer conditions if efficiency is low, for example, change transfer buffer, prolong transfer time.
	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, use non-denaturing gel systems.
	Concentration or affinity of used antibody is low.	Optimize concentration of the first antibody.
		For low-affinity antibodies, use Washing buffer without Tween 20.
	Peroxidase activity of the secondary antibody is low.	Dot different dilutions of the POD-conjugate onto a blotting membrane and detect directly. If no signal appears, use fresh POD-conjugate and test in the same way.
	Low activity of Detection reagent.	Check if the Luminescence substrate solution A and Starting reagent B were stored properly at +2 to +8°C.
		Make sure that the premixed Detection solution was not older than 1 week, and protected from light.
		Have all solutions been equilibrated to +15 to +25°C.
	Premixed Detection solution too old.	Prepare new Detection solution. Was the time between incubation and detection kept to a minimum?
	Incubation time too short.	Prolong the incubation time with primary antibody to overnight at +2 to +8°C.
	Detection time too short.	Prolong the detection time.
	Washing conditions not ideal.	Shorten washing times.
		Use Wash buffer without Tween 20.
	Insufficient amount of protein loaded.	Increase amount of protein applied onto the gel.
High background observed on blots.	High concentration of POD-conjugate.	Lower the concentration of POD-conjugate.
	Contamination of equipment or solutions.	Use clean equipment.
		Prepare fresh buffers.
	Contamination of membranes.	Use new membranes.
		Follow the membrane handling instructions, see section, **General Considerations**.
	Washing time too short.	Increase washing times.
	Long exposure time.	Shorten exposure time.

For nucleic acid analysis

Observation	Possible cause	Recommendation
No or weak signals, or low sensitivity.	Nucleic acid labeling reaction not optimized.	If you subject your hapten-labeled DNA/RNA to phenol extraction, the result is a partial loss of approximately 20% of the hapten-labeled DNA/RNA into the organic phase. This effect is further increased by addition of salt.
		When small amounts of template DNA are used, add 1 µl (20 µg) of inert glycogen* or 10 µl of 2 mg/ml of tRNA as carrier before precipitation to avoid loss of DNA. *You may also add a carrier during RNA precipitation, but this is rarely required due to the large amount of DIG RNA synthesized.*
	Hybridization conditions not optimized.	The conditions provided for hybridization and washing are highly stringent and work well for most applications. To enhance the sensitivity and reduce the time required for signal development, choose less stringent conditions: ▪ Perform hybridization down to +37°C with 50% formamide. ▪ Increase the salt concentration in the final washing step to 0.5x SSC. *RNA/RNA-hybrids tend to be more stable than DNA/DNA-hybrids and especially if using RNA probes, formamide and higher temperatures are used to avoid non specific hybridization.*
	Detection procedure not optimized.	Increase concentration of anti-hapten-POD.
		Prolong the detection time.
		Increase the amount of nucleic acid to the gel.
High background observed on blots.	Nucleic acid labeling reaction not optimized.	For colony hybridization, ensure that the labeled probe contains no plasmid sequences; gel-purify the insert twice.
	Hybridization conditions not optimized.	Increase the volume of prehybridization solution to allow filter to float freely.
		Decrease the amount of hapten-DNA/RNA probe in the hybridization solution.
		Make sure that filters do not dry out between prehybridization and hybridization.
	Hybridization conditions not optimized for colony and plaque hybridization:	Perform two prehybridization steps. ▪ After the first prehybridization, incubate the filters in 2x SSC, 0.1% SDS, 100 µg/ml proteinase K for 1 hour at +68°C. ▪ Wash filters 2 times in 2x SSC, 0.1% SDS at +68°C for 15 minutes before continuing with the second prehybridization.
	Detection procedure not optimized.	Decrease concentration of anti-hapten-POD.
		Increase volumes of the washing and blocking solutions, and duration of the washing and blocking steps.
		Shorten exposure time.

4. Additional Information on this Product

4.1. Test Principle

Detection of membrane-bound molecules is carried out by a variety of procedures.

- In protein analysis, the molecules of interest are usually detected with specific first antibodies, enzyme-labeled secondary reagents (antibody- or streptavidin-conjugates) and a suitable chromogenic substrate.
- A frequently used method in nucleic acid research is hybridization with radioactive probes and autoradiography.
- These detection systems, however, may be hazardous, time-consuming, or lacking in sensitivity. In addition, colors tend to fade with time and exposure to light.
- To overcome these problems, attempts have been made to use chemiluminescence for fast and sensitive detection of enzyme conjugates on blots.

The BM Chemiluminescence Blotting System is designed around peroxidase-labeled secondary reagents (antibodies, streptavidin; not included) and the substrate luminol.

- In the presence of hydrogen peroxide (H_2O_2), horseradish peroxidase (POD) catalyzes the oxidation of diacylhydrazides, such as luminol.
- An activated intermediate reaction product is formed, which decays to the ground state by emitting light.
- Strong enhancement of the light emission is produced by 4-iodophenol. This acts as a radical transmitter between the formed oxygen radical and luminol, see Figure 1.

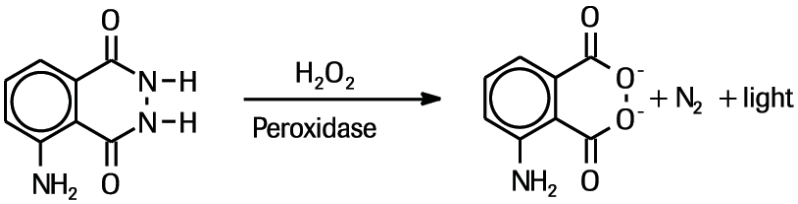

Fig. 1: Reaction mechanism

Labeling principle

Examples of chemiluminescent detection is shown in Figures 2 and 3.

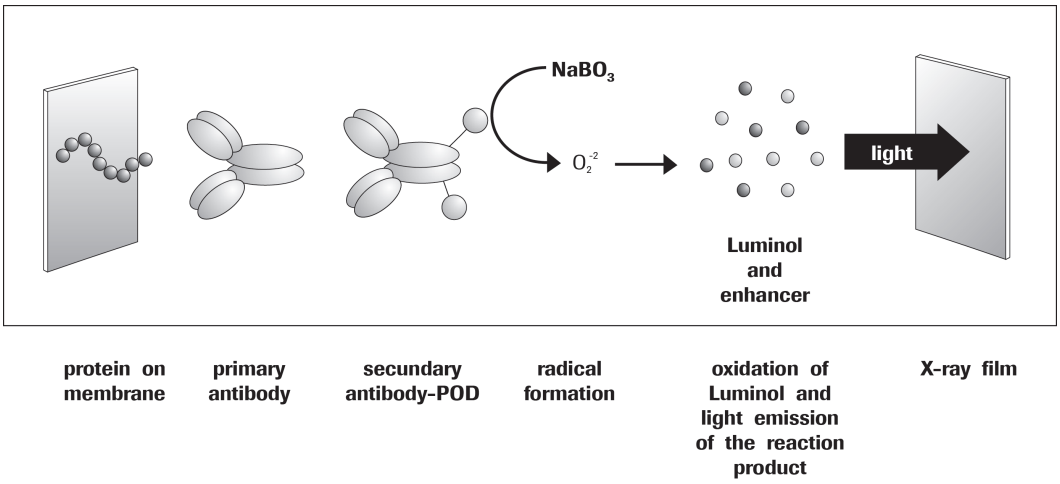

Fig. 2: Protein detection principle.

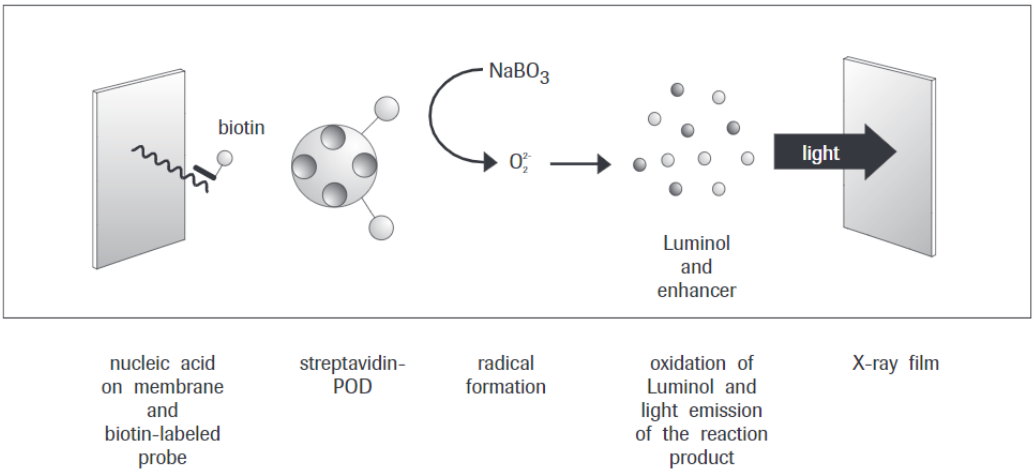

Fig. 3: Nucleic acid detection principle.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Poly(A)	100 mg	10 108 626 001
Poly(dA)	5 U, A_{260} units	10 223 581 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 l	11 666 681 001
Tris hydrochloride	500 g	10 812 846 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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