LIGHTwave™

ECL substrates for Western Blotting

Code	Description
LW0001	LightWave™ Western blotting substrate 10 mL
LW0002	LightWave™ Western blotting substrate 250 mL
LW0003	LightWave™ Plus Western blotting substrate 10 mL
LW0004	LightWave™ Plus Western blotting substrate 250 mL
LW0005	LightWave™ Max Western blotting high sensitive substrate 10 mL
LW0006	LightWave™ Max Western blotting high sensitive substrate 100 mL

About us

The GVS Group is one of the world's leading manufacturers of filters and components for applications in the Healthcare, Life Sciences, Automotive, Appliance, Safety, and Commercial & Industrial Filtration.
The Group's clear strategy towards internationalization, has led to the opening of 12 production facilities located in Italy, UK, Brazil, the United States, China and Romania, as well as offices in Russia, Turkey, Argentina, Japan, Korea. GVS currently have a workforce of over 2,700 people globally.
For 40 years, GVS has focused on innovation in its products range and production processes, constantly improving its development capacity to provide the best service and support for its clients
We offer a full range of branded products through a global network of dealers and distributors. We also make available all these capabilities on an OEM basis by working closely with companies around the world to provide state of the art materials solutions and/or turn-key final product solutions used in critical applications for the pharmaceutical, medical device, diagnostic, food & beverage and environmental monitoring markets.

All GVS substrates are protected by **US7803573**, **EP1962095**, **US7855287**, **EP1950207**, **US2012009603** (A1), **CA2742025**, **EP2405016**, foreign equivalents and pending patents.

LIGHTwave™

ECL substrates for Western Blotting

LightWave™ IS INTENDED FOR RESEARCH USE ONLY AND SHALL NOT BE USED IN ANY CLINICAL PROCEDURES OR FOR DIAGNOSTIC PURPOSES.

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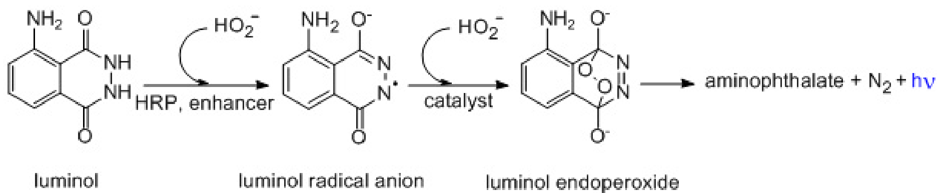
1. Introduction

LightWave™ detection reagents are non-isotopic, luminol-based chemiluminescence substrate, designed for the chemiluminescent detection of immobilized proteins and immobilized nucleic acids conjugated with horseradish peroxidase (HRP).
LightWave™ is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.



Storage/expiry

One year at room temperature (max. 25°C).

LightWave™ product line

Product	LightWave™	LightWave™ Plus	LightWave™ Max
Signal intensity	Medium	High	Ultra High
Signal duration	Medium	Extended	Short
Protein abundance	High	Medium	Ultra-low

2. Components and other materials required

Kit components

- Solution A: Luminol/enhancer solution (amber bottle)
- Solution B: Peroxide solution (white bottle)

Additional materials required

- Electrophoresis apparatus and buffers for SDS-PAGE
- Transfer apparatus for protein transfer from gel to membrane
- Nitrocellulose or PVDF membrane.
- Blocking buffer (Tris buffered saline (TBS) or Phosphate buffered saline (PBS) with 0.05-0.1% Tween-20 and 1-5% of a blocking reagent, such as bovine serum albumin (BSA), gelatin, casein, non-fat dry milk).
- Washing buffer (TBS or PBS with 0.05-0.1%).
- Primary antibody compatible with your application
- Secondary antibody, conjugated to Horseradish peroxidase (HRP) corresponding to your primary antibody
- CCD-based detection system or film.

3. Important Product Information

- Western blot results require an optimization of all the procedure steps and of all the reagents, such as sample loaded, gel type, transfer method, membrane type, blocking reagent, wash buffer, primary and secondary antibody concentration and incubation times.
- Use a sufficient volume of all solutions to avoid dry out of membranes.
- For optimal results, use a shaking or rocking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers, as it inhibits HRP.
- Always wear gloves when handling membranes and reagents.
- Do not use metallic forceps, which can cause speckling and/or high background due to rust contamination.

2. Components and other materials required

Kit components

- Separate protein samples via electrophoresis.
- Transfer proteins to membrane (PVDF or nitrocellulose) according to the manufacturer's instructions of your transfer device.
- Incubate the membrane in Blocking Buffer for 30 minutes to 1 hours with continuous agitation. A maximum blocking time of 2 hours at room temperature (RT) should not be exceeded. Blocking for too long can result in antigen masking and loss of protein.
- Sufficiently wash the blot with appropriate wash buffer.
- Incubate the blot with primary antibody in diluted blocking buffer solution or in wash buffer for 1 to 2 hours at RT. To increase sensitivity, try an overnight incubation at 4°C with agitation on a rocker.
- Wash the blot with appropriate wash buffer for three times for 5 minutes each with continuous agitation.
- Incubate the blot with secondary antibody solution in diluted blocking buffer solution or in wash buffer for 30 minutes to 1 hour at RT. Increasing the incubation time of the secondary antibody usually leads to higher background.
- Wash the blot with appropriate wash buffer from three to six times, for 5 minutes each, with continuous agitation.
- Perform the chemiluminescent detection.

IMPORTANT: It is crucial to optimize both primary and secondary Ab dilutions for best results with high signal and low background. Optimal Ab dilutions can be determined by Dot-Blot assay.

Product	Suggested antibody dilutions	
LightWave™	Primary Ab Secondary Ab	1:500 - 1:5,000 1:20,000 - 1:100,000
LightWave™ Plus	Primary Ab Secondary Ab	1:1000 - 1:15,000 1:25,000 - 1:150,000
LightWave™ Max	Primary Ab Secondary Ab	1:5000 - 1:100,000 1:100,000 - 1:500,000

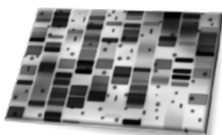
5. Chemiluminescent detection

- For reproducible performance, allow the detection solutions to equilibrate to RT before using.
- Prepare working solution (WS) by mixing properly each reagent in a 1:1 ratio. For best results, prepare WS immediately before use. Do not contaminate the solutions with the same pipette tips.
- Keep the membrane in wash buffer without Tween-20 until the incubation with LightWave™ WS.
- Place the membrane, protein side up, on a clear surface.
- Pipette the volume required directly onto the membrane and incubate for 1,5 min ensuring that the entire surface is covered.
- Use 0.1 ml of WS per cm2 of membrane.
- Acquire the signal with autoradiography film or imaging devices.
- For an unknown signal, try different exposure times.

6. Troubleshooting

High membrane background

High concentration of Ab. Further dilute primary and secondary Ab. Follow suggested Ab dilutions.
Inefficient blocking. Increase Tween-20 in TBS-T Buffer (0.1%÷0.5% v/v). Use 5% non-fat dried milk as blocking buffer if possible.
Insufficient washing. Increase both the volume, length and number of wash steps. Always use sufficient volumes to submerse the membrane.
Primary antibody is not specific for the protein of interest. Use monospecific or antigen affinity purified antibodies. Always incubate your primary antibody at 4°C overnight and not at room temperature. Reduce NaCl in TBS-T Buffer (100mM÷350mM). Use monospecific or antigen affinity purified Ab.
Non-specific binding of secondary antibody. Confirm the secondary is specific by omitting the primary and running a secondary only blot. If bands develop choose an alternative secondary antibody.
Incompatible blocking agent. Non-fat dry milk contains endogenous biotin and is incompatible with avidin/streptavidin systems. Substitute with 5% BSA.
Poor quality of antibodies. Quality and age of primary and secondary antibody may lead to background problems.
Poor handling of membrane. Be sure to handle the membrane only with clean plastic tweezers and non-powdered gloves.
Contaminated buffer solutions. Check buffers for particulate or bacterial contaminate. Replace old buffers.



Irregular black spots

Air bubble trapped in membrane. Remove air bubbles by gently rolling a clean pipette or a test-tube during sandwich assembling.
Unevenly hydrated membrane. Make sure that the membrane is fully immersed during washes and antibody incubations.
Contaminated equipment. Protein or pieces of gel remaining on the unit may stick to the membrane. Antibody can get trapped in the gel, and then are washed out poorly, resulting in intense localized signal.
Aggregation of blocking agent. When blocking agent is powder stir it over night at 4°C to make sure it is completely dissolved.
Interaction of the membrane with sample tray. Always use clean plastic trays to avoid any type of cross-reaction.

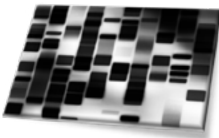
6. Troubleshooting

Formation of aggregates in HRP-conjugate. Filter secondary antibody solution through a 0.2 µm filter. Use fresh antibody.

No bands or weak bands

Excessive signal generated. The enzyme in the system depleted the substrate and caused the signal to fade quickly. Further dilute secondary Ab.
Inefficient transfer. Ensure that there is good contact between membrane and gel during sandwich assembling. High MW protein may require more time for transfer. Reduce voltage or time of transfer for low molecular weight proteins (< 10 kDa).
Antibodies may have lost activity. Perform a Dot Blot. Follow manufacturer's recommended storage and avoid freeze/thaw cycles.
Incorrect secondary antibody used. Confirm host species/Ig type of primary Ab.
Low protein-antibody binding. Reduce the number of washes to minimum. Reduce NaCl in TBS-T Buffer (100mM÷350mM).
Non-fat dry milk may mask some antigens. Decrease blocking time. Decrease milk percentage in Blocking Buffer or substitute with 5% BSA Blocking Buffer.
Sodium azide contamination. Make sure buffers do not contain sodium azide as this will quench HRP signal.
Contaminated stock solutions. Do not contaminate the chemiluminescent substrate stock solutions using the same pipette tip. Use new reagents.

Non-specific bands



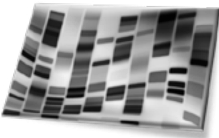
Aggregation of analyte. Increase amount of reducing agent to ensure complete reducing of disulfide bonds.
SDS interference. The presence of SDS may result in the development of unspecific bands caused by antibodies binding to the charged SDS molecules associated with the proteins. Wash thoroughly the membrane after transfer with water.
High protein concentration. A commonly seem effect is the diffusion of protein bands. Reduce the amount of protein initially loaded.
Primary antibody is not specific for the protein of interest. Use monospecific or antigen

affinity purified antibodies. Always incubate your primary antibody at 4°C overnight and not at room temperature. Reduce NaCl in TBS-T Buffer (100mM÷350mM). Use monospecific or antigen affinity purified Ab.
Non-specific binding of secondary antibody. Confirm the secondary is specific by omitting the primary and running a secondary only blot. If bands develop choose an alternative secondary antibody.

White bands or “ghost bands”

Excessive signal generated. Excessive antibodies or loaded protein can cause high levels of localized signal. This results in rapid consumption of substrate at this point. Since there is no light production after the completion of this reaction, white bands are the result. Try first to further dilute secondary antibody.

Uneven or jagged bands



Uneven gel run. Load all available wells. Empty wells can be loaded with sample buffer.
Voltage or current were too high during electrophoresis. Reduce voltage or current during electrophoresis.
Effects of high salt in samples. Reduce NaCl concentration in TBS-T Buffer (100mM÷350mM).

7. Selection Guide

Product	Competitors
LIGHTwave™	PIERCE™ ECL PLUS - THERMO SCIENTIFIC™ IMMOBILION® CLASSICO - MILLIPORE™ WESTERN LIGHTNING™ PLUS - PERKINELMER WESTERNBRIGHT™ ECL - ADVANSTA
LIGHTwave™ Plus	CLARITY™ - BIORAD SUPERSIGNAL™ WEST DURA - THERMO SCIENTIFIC™ AMERSHAM™ ECL PRIME™ - GE HEALTHCARE SUPERSIGNAL™ WEST PICO PLUS - THERMO SCIENTIFIC™ IMMOBILION® CRESCENDO - MILLIPORE™ WESTERNBRIGHT™ QUANTUM™ - ADVANSTA
LIGHTwave™ Max	CLARITY MAX™ - BIORAD SUPERSIGNAL™ WEST FEMTO - THERMO SCIENTIFIC™ AMERSHAM™ ECL SELECT™ - GE HEALTHCARE WESTERNBRIGHT™ SIRIUS™ - ADVANSTA WESTERN LIGHTNING™ ULTRA - PERKINELMER

For further information, visit

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