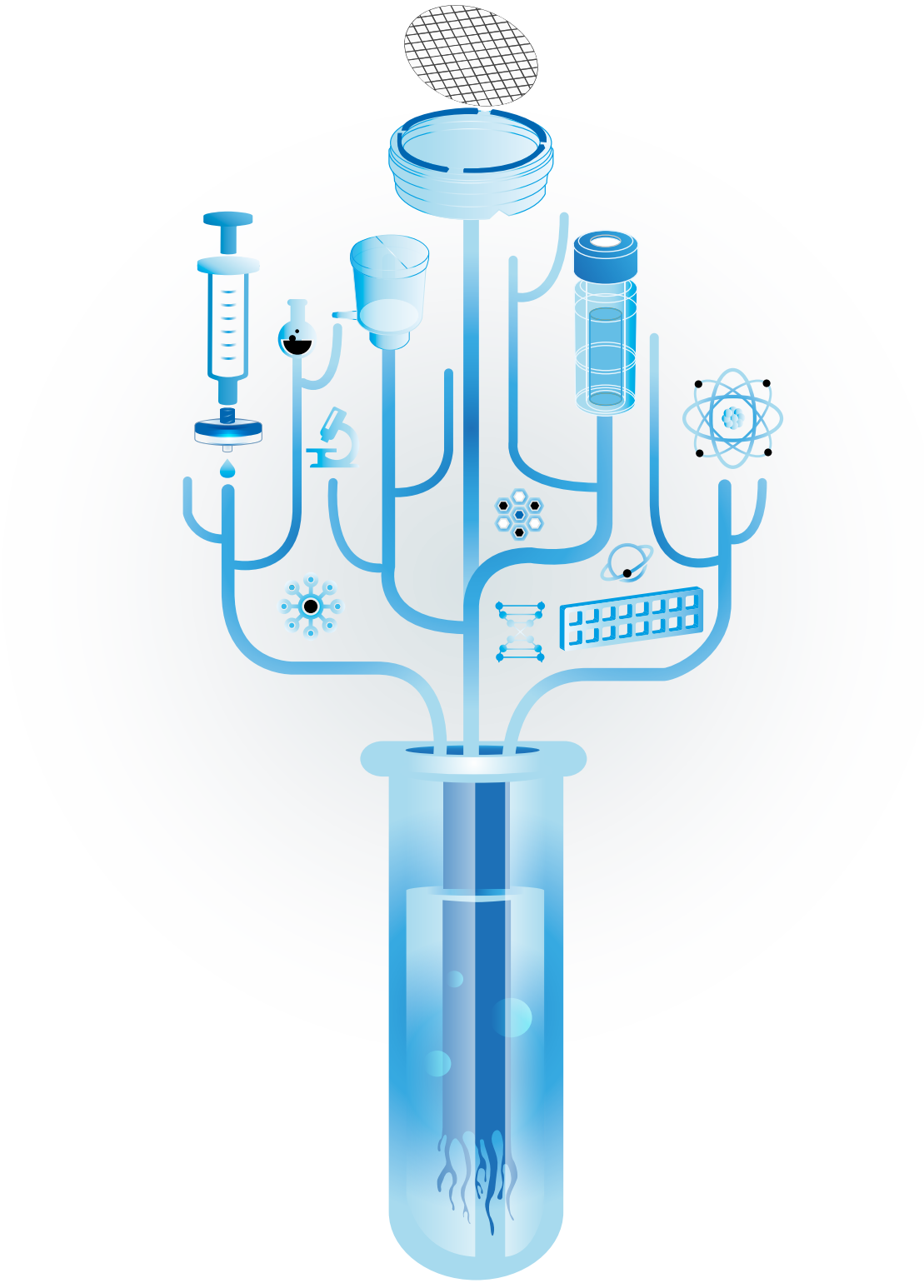
FILTER TECHNOLOGY

MOLECULAR BIOLOGY PRODUCT COLLECTION

GVS

FILTER TECHNOLOGY



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Molecular Biology

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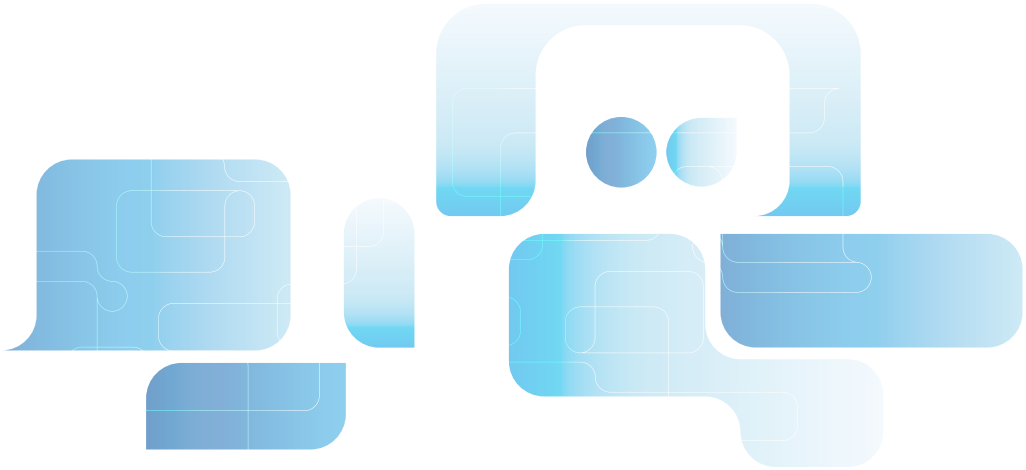
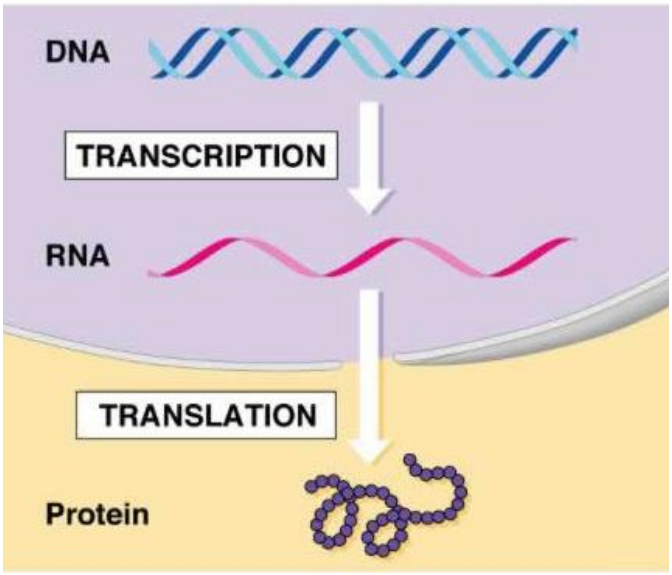


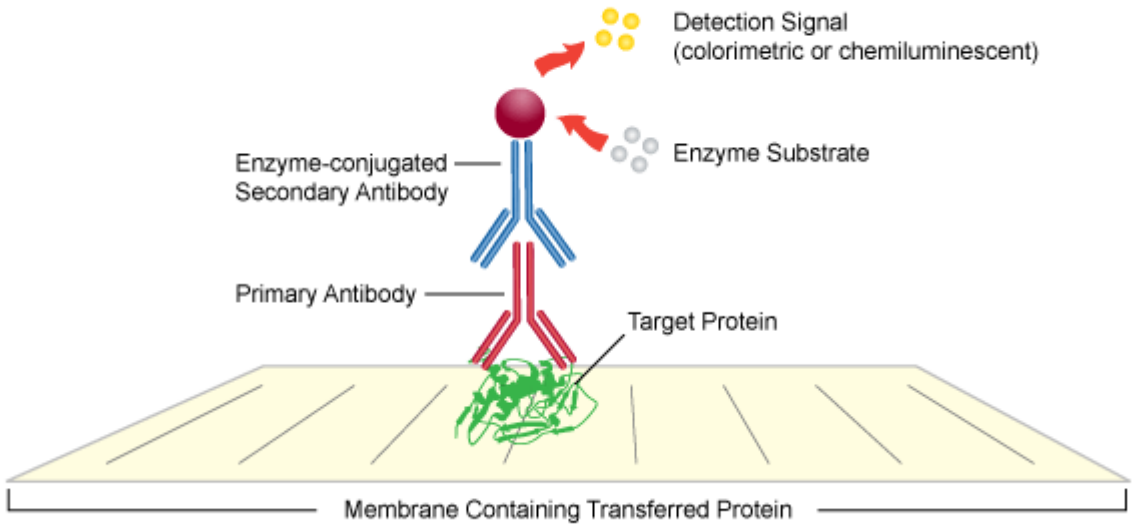
Molecular Biology Analysis

Molecular analysis studies subcellular components such as proteins and nucleic acids (DNA, RNA). These molecules can be detected by various blotting techniques. The sample of interest is separated according to size by electrophoresis through a gel. Molecules from the sample are transferred and bound to a microporous membrane Then, specific molecules of interest are detected using another molecule which specifically binds to the molecule of interest and can be detected by color, light or radioactivity.



Western Blot

Western blotting is a common and important technique used in molecular biology. It is used to detect a specific protein or protein fragment from a complex mixture such as a cell lysate, tissue extract, blood or serum sample or culture supernatants. The complex mixture is separated according to size by gel electrophoresis and then transferred to a membrane. A protein of specific interest is immunodetected using primary and secondary antibodies.



Western Blot Application Examples:

- **Protein expression and modification studies, may be quantitative;**
- **Amino acid analysis;**
- **Diagnostics development;**
- **Medical diagnosis such as for HIV and Lyme disease.**

Electrophoretic separation of proteins

Separation into polyacrylamide gel according to molecular wieght. In order to separate the proteins of lower molecular weight, use of more concentrated gel is required.

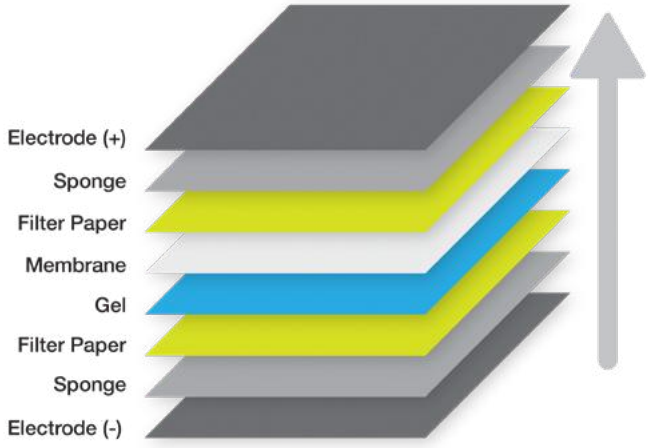
Transfer of proteins

Transfer from gel onto membrane followed by:

- Blocking;
- Applying a primary antibody specific for your protein of interest;
- Applying secondary antibody that will recognize the primary antibody.

Role of protein binding

Set up for transfer



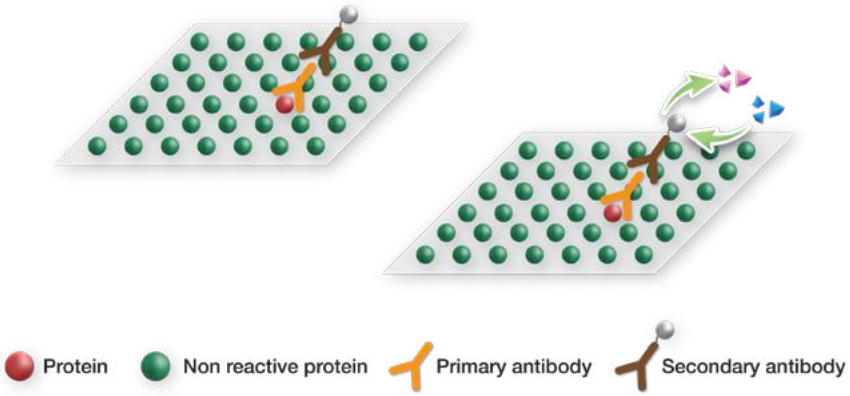
TYPICALLY TWO TYPES OF MEMBRANES:

- Nitrocellulose (hydrophilic)
- Polyvinylidene fluoride (hydrophobic)

Protein to membrane binding interactions:
hydrophobic, electrostatic, dipolar

Detection of proteins

Proteins can be detected by immunodetection methods which use enzyme conjugated/labeled secondary antibodies. When the enzyme substrate is added, a product is formed. This product can be detected by fluorescence, colormetrically, or by chemiluminescence. Enhanced chemiluminescence (ECL) produces light as a by-product when the substrate is catalyzed by the enzyme. This light is then captured on X-ray film or by a digital imaging system.



Transfer Membranes

Unlike many transfer membrane suppliers, GVS Filter Technology manufactures all of its nitrocellulose, PVDF membranes that we sell. Our transfer membranes are used in key research and testing done around the world.

PVDF

The PVDF-Plus is a naturally hydrophobic transfer membrane designed to deliver the highest binding capacity and lowest background in protein analysis applications.

Nitrocellulose

GVS Filter Technology manufactures two pure nitrocellulose transfer membranes. NitroBind is the classic unsupported pure nitrocellulose membrane used for all protein and immunoblotting applications. NitroPure is a supported pure nitrocellulose membrane combining the characteristics of nitrocellulose with the strength of nylon. It outperforms standard nitrocellulose in reprobing applications of DNA/RNA/Protein when extensive handling is required. Most other suppliers buy membranes from a manufacturer and sell them under their label. The chart below shows which of the market leaders actually make the transfer membranes they sell.

Membrane	Features	Benefits	Choose by Detection Systems	Choose by Procedures
NitroPure	Pure Nitrocellulose	Pure nitrocellulose is the membrane of choice for protein and immunoblotting techniques, as well as any other procedures that require optimum resolution. Binding Capacity - 100 µg/cm^2	Radiolabeled, Chromogenic and Chemiluminescent Detection Systems	Westerns Protein & Immunoblotting Northerns Southerns
NitroPlus	Supported Pure Nitrocellulose	Supported pure nitrocellulose is used in procedures requiring the highest sensitivities, low backgrounds and rigorous handling. The membrane can be reprobed many times. Binding Capacity 100 µg/cm^2	Radiolabeled Detection Systems Chemiluminescent and Biotinylated Detection Systems	Northerns Southerns Multiple Rehybridizations Colony and Plaque Lifts
PVDF-Plus	Hydrophobic PVDF Membrane	Hydrophobic PVDF membrane is desigened for protein sequencing, western trasfers and mino acid analysis. Binding Capacity - 125 µg/cm^2	Chemical compatibility allows the use of all commonly used stains	Western Transfers Protein Sequencing Amino Acid Analysis

Selecting a Membrane

Selecting the appropriate membrane is critical to the success of a nucleic acid or protein transfer procedure.
GVS Filter Technology manufactures many types of membranes for hybridization technology, each exhibiting different performance characteristics which can directly affect the outcome of a specific technique. Below are some of the more frequently performed procedures and features of hybridization membranes.

Rehybridizations

GVS Filter Technology manufactures membranes recommended for rehybridization procedures: Magna Nylon, NitroPlus Nitrocellulose supported and NitroPure, a supported pure nitrocellulose. NitroPlus Nitrocellulose supported membranes can be most frequently reprobed. On nylon membranes, the number of reprobing steps is a function of the amount of hydrolysis to which the membrane is exposed during the protocol, and the additive effects of hot water, sodium hydroxide and an acidic environment. Sodium hydroxide solutions deteriorate the nylon matrix and are not recommended in procedures where reprobing steps are required. The polyester support web used in manufacturing NitroPure allows the membrane to be reprobed several times. Because the binding capacity of nitrocellulose is less than that of nylon (100 µg/cm2 vs. 400 µg/cm2), the potential number of rehybridizations is fewer. See pages 105-107 for more details.

UV Crosslinking

For covalent binding of nucleic acids to a transfer membrane, GVS Filter Technology membranes can be UV Crosslinked by following the manufacturer's instructions. It is particularly recommended when working with short fragments, small samples, or low numbers of base pairs, because of the improved resolution this technique offers.

Protein Blotting

NitroPure nitrocellulose and PVDF-Plus membranes are recommended for use in protein blotting. Nitrocellulose membranes are able to be more thoroughly blocked, reducing the high background potential associated with protein blotting. PVDF membranes are more resistant to the harsh chemicals used in Edman degradation.

Alkaline Blotting
For more rapid transfers, an alkaline blotting procedure can be used with MagnaProbe or MagnaCharge membranes. Alkaline blotting is not recommended when reprobing is required. Please see page 125 for more details.

Staining Procedures
NitroPure, NitroPlus and PVDF-Plus membranes are recommended for procedures that require a staining step with India Ink, Coomassie Blue, Colloidal Gold, or any other commonly used stain. Nylon membranes irreversibly bind many stains.

Reducing Backgrounds
There are many sources of background problems, or low signal-to-noise ratios. Some of the most common include: contaminated probes, contaminated hybridization solutions, and incorrectly chosen stringency levels. Nonfat milk should not be used as a blocking agent as it may increase nonspecific binding. GVS Filter Technology membranes are all manufactured by strict quality control procedures, ensuring a uniform membrane with consistently low backgrounds. Please refer to pages 91-93 for more details.

Troubleshooting Common Blotting Problems
Many blotting problems can be eliminated by observing the following recommendations.
Blotchy or incomplete transfers are caused by poor contact between the gel and the membrane. Even after careful smoothing of the membrane to the gel, incomplete degassing of transfer solutions can cause air pockets to form. Evolving gas from Tris or, in the case of protein transfers, methanol, can disrupt the tight contact necessary between the membrane and the gel for successful transfers. Smeared or skewed bands are often caused by uneven contact between the gel and the membrane, or the membrane and the chromatography paper. To avoid this problem, roll a pipet down the membrane after it has been applied to the gel, and once again over the chromatography paper after it has been applied to the membrane. Do not move the membrane until the transfer is complete, as this will cause smearing.

Protocols for Protein Applications

Gel Preparation

Western (Protein) Blotting
With NitroPure and NitroPlus Nitrocellulose Membranes
Gels should be stained after transfer with Coomassie Blue, Fast Green, Amido Black, or any other appropriate stain.* Soak the gel for 1 hour in a transfer buffer made of: 25 mM Tris-HCl/pH 8.0, 0.15 M glycine, 20% methanol. *GVS Filter Technology does not recommend staining before transfer. Proteins may precipitate in the membrane and not be able to transfer.

Transfer Membrane Preparation
Completely soak the membrane in deionized water, and then in transfer buffer.

Electroblotting
Assemble the membrane and gel in the electroblotting unit. Place the membrane on the anode (positive) side of the gel. Transfer according to manufacturer's instructions. Remove and wash thoroughly with transfer buffer.

Capillary Blotting
Prepare gel assembly by the method of Southern (see page 115). Transfer for 2 hours to overnight. Use transfer buffer of 10 mM Tris-HCl/pH 7.5. After the transfer step, determine transfer efficiency by staining the blot or gel by standard methods.

Blocking Procedures
Step 1: First Wash
Block the blot in PBS buffer (0.9% NaCl, 10 mM sodium phosphate/pH 7.2) containing 5% BSA, Tween 20 or high purity gelatin for 1 hour, with gentle agitation.
Step 2: Primary Antibody Binding
Remove the PBS buffer solution from blot completely. Dilute the first antibody in 50 ml of fresh PBS buffer solution. Incubate the blot in the PBS blocking buffer/antibody solution for 1 hour at 37°C with gentle agitation. Use a ratio of 5-10 ml of solution to 100 cm2 of membrane.
Step 3: Second Wash
Wash the membrane in 100 ml of fresh PBS buffer solution (without antibody) with 0.1-0.3% Tween-20. Agitate in a shaker for 5 minutes. Repeat the wash step 2 times. (Note: Increasing the number of short washes reduces the potential for high backgrounds).

Detection
Thoroughly remove the PBS buffer solution and overlay the blot with an antispecies (second) antibody, or with protein A (radiolabeled or enzyme linked) for 1-2 hours at room temperature with gentle agitation. The final concentration of radiolabeled second antibody solution should be 1-2x 105 dpm/ml of PBS buffer solution. Enzyme-linked second antibody solutions should be made at a 1:1000 titer in PBS buffer solution. Repeat the wash step described in the procedure above.

Signal Development
The choice of signal development method is dependent on the type of probe used. Radiolabeled probes are developed and quantitated by autoradiography. Enzyme-conjugated labels (horseradish peroxidase or alkaline phosphatase) are developed and quantitated with the appropriate substrate solution.

Probe Removal (Stripping)
Do not allow the filter to become dry, or irreversible binding of the probe will result.
Wash the membrane at 60°C for 30 minutes in 0.05 M sodium phosphate/pH 6.5, 10.0 M urea, 0.1 M 2-mercaptoethanol, or wash the membrane in 0.2 M glycine-HCl, 0.5 M NaCl for 5 minutes. Rinse in 0.1 M NaOH or 0.5 M Tris for 10 minutes.

Western (Protein) Blotting from a SDS-PAGE System with PVDF-Plus

PVDF-Plus transfer membranes are an alternative to nitrocellulose membranes in Western blotting applications. They offer greater mechanical strength, high protein binding capacity and compatibility with protein staining and immunoblotting protocols. Its chemical stability allows use of a wide range of solvents for rapid destaining.

Tank Electroblotting
After electrophoresis, place the SDS-PAGE gel in a transfer buffer of 25mM Tris/192mM Glycine in 15% methanol*, pH 8.2, to equilibrate for 15-20 minutes before blotting. Size and cut the PVDF-Plus membrane to fit the SDS-PAGE gel. Pre-wet the membrane in 5-10 ml of 100% methanol for 5 seconds. Place the membrane in 500 ml of water for 5 minutes to remove the methanol. Equilibrate with transfer buffer (500 ml, as described above) for 10-15 minutes prior to use in blotting. The PVDF-Plus membrane must be kept wet at all times. If the membrane dries out, re-wet in methanol and water as described above, and proceed with the transfer. Assemble the blotting cassette as follows: Insert one half of the plastic cassette in a large dish containing transfer buffer. Place a piece of moistened filter paper on the cassette. Carefully put the gel onto the wet filter paper, and place the wet PVDF-Plus membrane onto the gel, removing any trapped air bubbles. Overlay a second sheet of moistened filter paper on top of the PVDF-Plus membrane, again removing any trapped air bubbles. Place a porous foam sheet on top to complete the sandwich and ensure uniform membrane contact with the gel, and insert the assembled cassette into the blotting apparatus. Be sure that the membrane side of the assembly faces the anode electrode so that the transfer from the gel to the membrane will take place. Fill the electroblotting apparatus with 4-5 liters of transfer buffer and connect the cooling coil to a suitable circulating cooling water bath. Transfer proteins from the SDS-PAGE gel to the PVDF-Plus membrane at 70v for 1-2 hours. After transfer, stain the membrane with Amido Black or Coomassie Blue for 10 minutes followed by rapid destaining in 50% methanol, 10% acetic acid for 10 minutes, followed by a wash in distilled water.

*NOTE: Too high a concentration of methanol can lead to aggregation of high molecular weight proteins in the gel preventing transfer.

Semi-Dry Electroblotting
After electrophoresis, place the SDS PAGE gel in transfer buffer for 15-20 minutes to equilibrate before blotting. See the semi-dry electroblotter manufacturer's instructions for details on assembly and recommended buffers.
Transfer times are dependent on the size of the proteins and percentage of gel used. In most cases, complete transfer is achieved in 30 minutes. Transfer times should never exceed one hour.

Protein MW Range
(Daltons) Estimated Transfer Time
50,000 15 minutes
50,000 - 200,000 30 minutes
Up to 250,000 40 minutes
Note: Be sure to remove all trapped air bubbles to avoid bald spots in the blotted membrane.
Also, when transferring from thin polyacrylamide gels, place the wetted transfer membrane onto the gel while still on the electrophoresis plate. Invert the plate and carefully place the membrane/gel sandwich onto wetted filter paper, then remove the glass plate and continue.

Storage
Membrane blots can be dried and stored at 4°C for use at a later date. Rewet the membrane by prewetting it in a small volume of 100% methanol for 2-4 seconds then placing it in a large volume of deionized water to remove the methanol or, if exposure to methanol is to be avoided, the membrane blot can be taken directly to protein background “blocking” and then air dried. This coats the membrane with hydrophilic protein and allows easy rewetting in antibody incubation solution.
Using this method requires that the membrane strips be stored at 4°C. The blots are stable for up to 1 year when stored dry.

Immunostaining
Begin by incubating blots in a protein blocking solution of 5% BSA in TBS for 1 hour at 37°C. After incubation with the protein blocking solution, the blots should be washed for 5 minutes in a wash solution of 0.1% (w/v) BSA in TBS. Repeat the wash cycle 3 times. Incubate with the primary monoclonal antibody, which has been diluted 1:1000 with an antibody incubation solution of 1% BSA, 0.05% Tween-20 in TBS, for 2 hours at room temperature with gentle agitation. Repeat the wash step and incubate with one of the following enzyme-conjugated second antibody working solutions for 2 hours at room temperature.
Horseradish Peroxidase (HRP) conjugated rabbit anti-mouse IgG (1:500 dilution in 1% BSA, 0.05% Tween-20 in TBS). Alkaline Phosphatase conjugated goat anti-mouse IgG (1:500 dilution in 1% BSA, 0.05% Tween-20 in TBS).
Colloidal Gold labelled goat anti-mouse IgG (1:100 dilution in 1% BSA, 0.05% Tween-20 in TBS overnight at room temperature). Repeat wash step and then incubate with the appropriate substrate system to develop the colored reaction product. The development of the color can take 10-20 minutes at room temperature. Horseradish Peroxidase Substrate development is stopped by rinsing in water followed by drying the membrane. The developed blots should be stored dry, and protected from light.

Enzyme Conjugate Substrates
Horseradish Peroxidase
AEC (3-amino-9-ethylcarbazole)
0.8 mM AEC
29 mM sodium acetate
0.2 mM thimerosol
0.02% hydrogen peroxide
4CN (4-chloro-1-napthol)
DAB (3, 3’-diaminobenzidine)

Alkaline Phosphatase
0.56 mM 5-bromo-4-chloro-3-indolyl phosphate (BCIP)
0.45 mM NitroBlue tetrazolium (NBT) in 10 mM Tris/HCl pH 9.5
Colloidal (silver enhancement)
77 mM hydroquinone
5.5 mM silver lactate in 100 mM citrate buffer pH
3.85 (make reagent in a darkened container).
After silver enhancement is complete, soak the blot in fixing solution, for 5 minutes followed by a rinse in water.

Storage
Store blots dry. Avoid fading by protecting the blot from light.

Protein Staining
Low Sensitivity Detection (.001-.005 µg/band).

Ponceau-S Dye
After blotting, rinse PVDF-Plus with DI water. Stain with 0.2% of Ponceau-S for 1 minute. Rinse briefly with DI water to remove excess stain. To remove stain from protein bands, rinse briefly (<5 minutes) in 0.1 N NaOH. This may be less successful with basic proteins and could lead to protein loss from the membrane.

Amido Black or Coomassie Blue
Membrane strips should be stained in Amido Black or Coomassie Blue for 10 to 15 minutes, followed by destaining for 10 to 15 minutes in 45% methanol/7% acetic acid for Amido Black and 50% methanol/10% acetic acid for Coomassie Blue. Place the blots in 90% methanol for 1 to 2 minutes to remove any residual stain. High Sensitivity Detection 0.005-0.1 ug/band.

Troubleshooting Guide and Application Tips

Problems and Solutions

Unsuccesful Rehybridizations

My membrane is deteriorating during the rehybridization procedure?

If so, what type of membrane are you using? GVS Filter Technology manufactures one type of membrane recommended for rehybridization procedures: NitroPlus, a supported pure nitrocellulose. A more resilient membrane during applications requiring multiple reprobes. Nitropure (a supported nitrocellulose) was developed for this reason. The polyester support web used in manufacturing NitroPlus allows the membrane to be reprobed several times.

My application demands an extensive number of reprobes and I'm losing signal?

If so, what type of membrane are you using? Because the binding capacity of nitrocellulose is less than that of nylon (100 µm/cm2 vs. 400 µm/cm2), the potential number of rehybridizations is fewer as compared to nylon membranes. The number of reprobing steps is a function of the amount of hydrolysis to which the membrane is exposed during the protocol, and the additive effects of hot water, sodium hydroxide and an acidic environment.

My probe is not stripping from the membrane, how should I change my procedure?

Did you let the membrane dry after the initial probe was applied? Drying causes irreversible binding of DNA to microporous membranes. If this has occurred, look through the helpful tips listed below.

My probe won't strip from the membrane, how can I rescue this blot?

Try preparing a new probe and using a different detection protocol. For example, if you prepared a biotinylated probe and detected with a streptavidin conjugate, omit the biotin-streptavidin step during rehybridization by using a directly conjugated probe, such as an alkaline phosphatase conjugated probe. If you used a radioactive probe, use a chemiluminescent system to detect after the next hybridization (or vica versa). If you have enough time and are using radioactive probes (e.g., pgs 106-107), simply let your first probe decay before the second round of hybridization.

Signal Problems

The nucleic acid did not transfer completely to the membrane, what should I do?

Blotchy or incomplete transfers are caused by poor contact between the gel and the membrane. Even after careful smoothing of the membrane to the gel, incomplete degassing of transfer solutions can cause air pockets to form. Evolving gas from Tris or, in the case of protein transfers, methanol, can disrupt the tight contact necessary between the membrane and the gel for successful transfers.

My Signal is low, what are the common reasons for this?

When you have low signal, it is best to check your reagents by performing extra controls. The most common reason for poor signal is a bad probe. Prepare a new probe and perform a dot blot comparing the old and new probes. Do you see a difference between the probes? Even nonradioactive probes can deteriorate during storage. Is the signal weak for the new probe as well? Then your detection enzymes may be bad or the reagents used to prepare the probe are bad. You might also blot a small amount of unlabeled complementary DNA and hybridize to the new probe. Are you seeing signal from the blotted probe but not the hybridized DNA? If so there could be a problem with your hybridization protocol, such as the wash temperature or your buffers. If you're using nonradioactive detection methods, test your enzymes and substrates as well.

Background Problems

Everything was working fine and now suddenly I have high backgrounds,Why?

Did you make up a new probe? If so, was there adequate separation of the unincorporated label from the incorporated? Are you using old solutions? There may be contamination. Usually in these cases it is best to prepare new solutions, new probes and use new reagents. This is often the fastest way to get your system working again.

Miscellaneous

My membrane changed color during my blotting procedure, should I be concerned?

No. Slight color changes in GVS Filter Technology new positively charged membranes are expected and have no effect on results.

These color changes will vary according to the blotting procedure used and the pH of solutions. GVS Filter Technology uses this color change to ensure quality during the manufacturing procedure.

High Backgrounds

Poor agitation during prehybridization and hybridization steps can lead to insufficient blocking of the entire membrane. Due to the strength of the internal support web, NitroPlus can withstand higher levels of agitation without tearing or ripping. Incorrect probe concentration can occur when using dextran sulfate in hybridization or prehybridization solutions. Dextran sulfate causes the effective concentration of the probe to increase because it excludes the probe from the volume of solution the dextran sulfate polymer occupies. When using dextran sulfate, lower the probe solutions to less than 10 ng/ml of the solution. When not using dextran sulfate, maintain the optimum probe concentration at 25-40 ng/ml of solution. Residual agarose on membranes can cause a fuzzy background to appear on blots. Be sure to wash nylon membranes with 5 x SSPE at 60°C, after the immobilization step. Due to the strength of the membrane, supported membranes (NitroPlus) can be more easily washed without tearing or ripping.

Troubleshooting Gel Casting Procedures

Troubleshooting blotting problems begins with the correct gel casting procedures. Skewed, streaked, incomplete, or nonuniform transfers can be the results of poorly cast gels. The following recommendations are made for setting up the gel.
Gels greater than 4mm thick can interfere with the free transfer of nucleic acids.
Be sure that the gel tray is level before casting the gel. If the surface is not level, non-uniform transfers may result. Maintain a gel casting temperature of 55-70°C degrees, and be sure that the gel particles are completely dissolved. Undissolved agarose particles can result in streaked or skewed bands. Immediately after gel casting and solidification, submerge the gel slab in electrophoresis buffer. This will prevent the formation of an impermeable “skin” over the surface of the gel which can inhibit transfer of nucleic acids from the gel. After setting up the blotting assembly, be sure to:

- Invert the gel so that the underside of the gel is the side in contact with the membrane.
- Allow the transfer solutions enough time to “breathe,” so that they may degas completely. Incompletely degassed transfersolutions evolve gas after the blotting assembly is set up, and can cause air bubbles between the membrane and gel that can impede the transfer of nucleic acids.

Probe Related Background Problems

While there are several ways to decontaminate probe solutions, the following methods are two of the most efficient. The second method can be rapidly performed with minimum effort.
Method 1: Phenol/Chloroform extract the probe to remove unincorporated nucleotides, proteins, and other contaminants.
Method 2: Clean the probe by adding a small volume of the hybridization buffer to the probe and filtering it through an Abluo 25AS low protein binding cellulose acetate syringe filter. Contaminants in the probe solution will be held back by the 0.2µm filter with no probe loss caused by nonspecific binding to the filtration membrane. Probe length is also a factor contributing to background levels seen on transfer membranes. Between 250-800 base pairs is the recommended optimum length of a probe; probe lengths smaller or larger than this can lead to a low signal-to-noise ratio. Probes smaller than 250 base pairs often bind poorly and may require less stringent hybridization and wash procedures. Probes larger than 800 base pairs may contain a wider variety of size classes, which can lead to extraneous binding to the transfer membrane.

Hybridization Solution Related Background Problems

Contaminated hybridization solutions are another common source of background problems. Hybridization solutions should be filtered with a pure cellulose acetate Abluo 25AS syringe filter, to remove contaminants.
Additionally, all solutions and buffers should be made fresh before each transfer with sterile, double-distilled, deionized water, and very high grade reagents. After fresh buffers are made, they should be filtered with an Abluo 25AS syringe filter to ensure that no contaminants remain in the solution. Formamide-based hybridization solutions are a frequent source of background noise, and the formamide must be freshly made and deionized.

Optimized Blocking Solutions

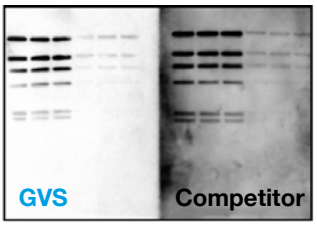
A concentration of 5-7 x Denhardt’s solution is recommended for use with nylon membranes. Exceeding this level can lead to quenching of the signal.

Backgrounds Associated with Reprobing

A follow-up autoradiograph after probe removal is strongly recommended to determine if the probe has been fully stripped. Otherwise, backgrounds can appear in blots that have not been fully erased.

Pure Nitrocellulose

NITRO Pure
Unsupported
Nitrocellulose



GVS Nitrocellulose Pure Transfer Membrane is the membrane of choice for all protein or immunoblotting applications. The high sensitivity of GVS Nitrocellulose Transfer Membrane ensures excellent results in all transfers, especially in protein blotting. Unlike PVDF, nitrocellulose wets out naturally, does not require methanol, and will not turn hydrophobic during the transfer process.
Nitrocellulose is very easily blocked and does not need the many blocking steps required with PVDF.
Excellent results will be obtained with all detection systems: antibody/antigen, radiolabeled, biotinylated, and chemiluminescent, giving you a great amount of flexibility in designing your procedure.

Supplied in various porosity and format.

Features & Benefits

- For procedures that require optimum resolution
- Membrane of choice for protein or immunoblotting applications
- Low background, easily blocked
- BSA binding capacity up to 100 µg/cm^2
- Wets out naturally
- Compatible with all detection systems

Typical Applications

- Western Blotting
- Protein & immunoblotting
- Northern Blotting
- Southern Blotting
- Dot/slot blotting
- Radiographic, chromogenic and chemiluminescent detection systems

Product	Competitors
NITRO Pure Unsupported Nitrocellulose	Amersham HyBond-C - BioRad Nitrocellulose - Millipore Immobilon-NC Plus - Shleicher & Shuell (S&S) Protran

Ordering information

Dimensions (mm) Packaging	70x84 mm 10/pk	100x100 mm 10/pk	150x150 mm 5/pk	200x200 mm 25/pk	200x3000 mm 1/pk	300x3000 mm 1/pk
Pore sizes 0.22 µm	1213991	1213999	1215463	1215392	1215469	1215458
0.45 µm	1213888	1213314	1215476	1221976	1215483	1215471

Supported Nitrocellulose

NITRO Plus Supported Nitrocellulose

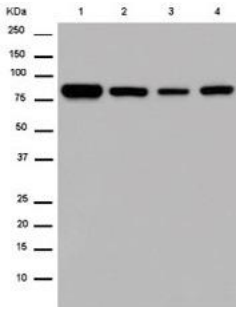
GVS Supported NitrocelluloseTransfer Membrane combines the binding characteristics of nitrocellulose membrane with the strength of nylon membrane. It can be easily used in any protocol utilizing unsupported nitrocellulose transfer membrane. Supplied in various porosity and format

Features & Benefits

- Supported for procedures requiring rigorous handling
- Strong - will not curl, bend or crack after baking
- High sensitivities, low backgrounds
- Multiple reprobings
- BSA binding capacity up to 100 µg/cm^2
- Triton Free

Typical Applications

- Northern Blotting
- Southern Blotting
- Multiple re-hybridizations
- Colony/plaque lifts
- Dot/slot blotting
- Radiographic detection systems
- Chemiluminescent detection systems
- Biotinylated detection systems



All lanes : Anti-Furin antibody [EPR14674] (ab183495) at 1/5000 dilution

Lane 1 : HepG2 whole cell lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : U87-MG whole cell lysate
Lane 4 : Caco-2 whole cell lysate
Lysates/proteins at 20 µg per lane.
Secondary
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution
Predicted band size : 87 kDa

Product	Competitors
NITRO Plus Supported Nitrocellulose	Amersham HyBond-C Extra - Amersham HyBond-C Super Biorad Supported Nitrocellulose

Ordering information

Pore sizes	Dimensions (mm) Packaging	70x84 mm 10/pk	100x100 mm 10/pk	150x150 mm 5/pk	200x200 mm 5 /pk	200x3000 mm 1/pk	300x3000 mm 1/pk
	0.22 µm		1214560	1212669	1212689	1212690	1212632
	0.45 µm	1214978	1213943	1212596	1212597	1212602	1212590

Polyvinylidene Fluoride PVDF

PVDF *Pure*
Unsupported PVDF

GVS PVDF is a naturally hydrophobic, unsupported transfer membrane. It has a high binding capacity, which prevents protein from passing through the membrane, and a low background that provides for an excellent signal-noise ratio. It also has exceptional tensile strength, preventing it from cracking, tearing, breaking or curling. This membrane also has broad chemical compatibility, which is important when used with common stains such as Amido Black, Colloidal Gold, Coomassie Blue, India Ink and Ponceau-S. GVS PVDF will not degrade, distort or shrink when a high concentration of methanol is used for destaining.

Its exceptional strength, high binding capacity and chemical compatibility make GVS PVDF ideal for use in Western blotting, immunoblotting, and solid phase assays and plaque lifts.

Features & Benefits

- Superior strength: Can withstand aggressive handling or be used with automated equipment without breaking or tearing
- Low extractables: Ensures tests will be clean with consistent results
- Exceptional sensitivity: Detects low-level components
- Hydrophobic: For high protein binding
- Lot-to-lot consistency: Quality checks ensure consistent binding for dependable results every time
- BSA protein binding capacity : 125 µg/cm^2
- High range of chemical: Resistant to most commonly used chemicals compatible with chemically aggressive solvents

Typical Applications

- Western blotting
- Immunoblotting
- Solid phase assays
- Amino acid or protein analyses

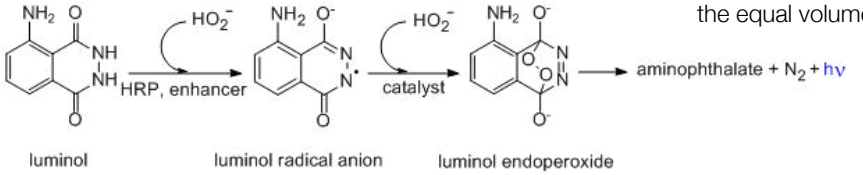
Product	Competitors
PVDF *Pure* Unsupported PVDF	Millipore Immobilon-P - Amersham HyBond - BioRad PVDF Shleicher & Shuell (S&S) Westran

Ordering information

Pore sizes	Dimensions (mm) Packaging	70x84 mm 10/pk	100x100 mm 10/pk	150x150 mm 5/pk	200x200 mm 5/pk	200x3000 mm 1/pk	300x3000 mm 1/pk
	0.22 µm	1214588		1215037	1215032	1214726	1214429
	0.45 µm	1213992	1212644	1212636	1212637	1212783	1212639

Introduction

LightWave is our product line of ECL HRP substrates for Western blotting. Our double enhancer proprietary technology allows for modulation of signal intensity and signal duration. Each LightWave substrate is at the top of its respective market segment regarding performance/price ratio.
All LightWave substrates are:
- Compatible with all chemiluminescence imagers and X-ray film detection
- Optimized for attaining low background and high signal to noise ratio
- Stable for 1 year at RT



All GVS substrates are protected by **US7803573**, **EP1962095**, **US7855287**, **EP1950207**, **US2012009603** (A1), **CA2742025**, **EP2405016**, foreign equivalents and pending patents.
LightWave™ detection reagents are non-isotopic, luminol-based chemiluminescence substrate, designed for the chemilumine-scent 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.
Chemiluminescent substrates for horseradish (HRP) are two-component systems consisting of a stable peroxide solution and an enhanced luminol solution. To make a working solution, the equal volumes of the components are mixed together.

Storage/expiry

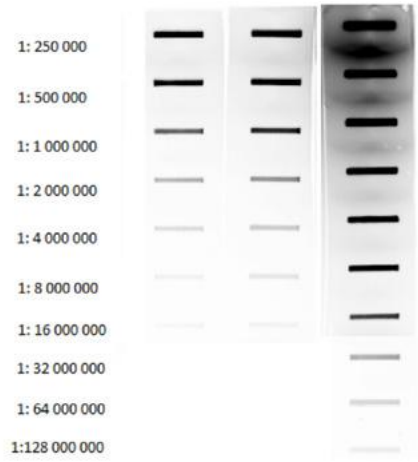
One year at room temperature (max. 25°C).

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

Overview

HPR - Antibody dilutions



LightWave - Low picogram detection level
LightWave Plus – Mid femtogram detection level
LightWave Max – low femtogram detection level

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

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

GVS LightWave Substrates

GVS Lightwave

LIGHTwave™

Features

- Low picogram detection
- Ideal for routinary analysis
- Working solution stable for at least three days
- The best entry level ECL substrate on the market
- Signal duration 5 hours
- Stable for 1 year at RT

Sample: Two-fold dilution series of Hela whole cell lysate (abcam®) from 5 µg to 0.078 µg of total protein
Primary antibody: Rabbit-anti Human HDAC-1 (abcam®) 1:2000
Secondary antibody: Goat anti-rabbit IgG HRP (2mg/mL) (abcam®) 1:20000
Imaging: ImageQuant™ LAS 4000 (GE Healthcare)
Exposure time: 180 seconds

Quick start protocol

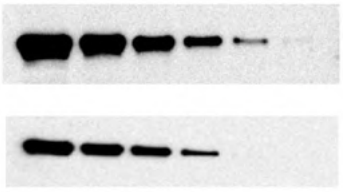
- Perform electrophoresis, membrane transfer and antibody incubation and washes
- Prepare Lightwave™ ECL substrate by mixing equal volumes of the two solutions
- Apply Lightwave™ chemiluminescent substrate to the membrane (1 mL per 10 cm^2 of the membrane), incubate 2 minutes with the substrate
- Expose the substrate-treated membrane using a chemiluminescence imager or X-ray film

GVS LightWave vs Competitor Signal duration

Comparison of signal intensities at time points up to 20 hours post substrate addition. Exposure time is 180 seconds for each time point (0-2-5-8 hours).

Code	Description
LW0001	LightWave™ Western blotting substrate 10 mL
LW0002	LightWave™ Western blotting substrate 250 mL

GVS Lightwave Plus

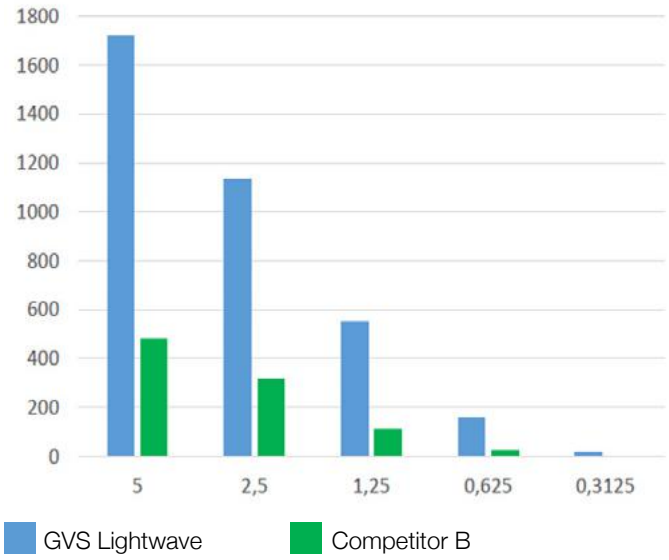


Lightwave Plus

Competitor

Western blotting detection of HDAC-1 on Hela cells
Hela cell lysate from 5 to 0,078 µg
Ab 1° Rabbit anti HDAC1 1:5000
Ab 2° Goat anti rabbit 1:50000
Exposure time: 3 minutes
Imager: LAS4000 (GEHC)

Signal to noise ratio



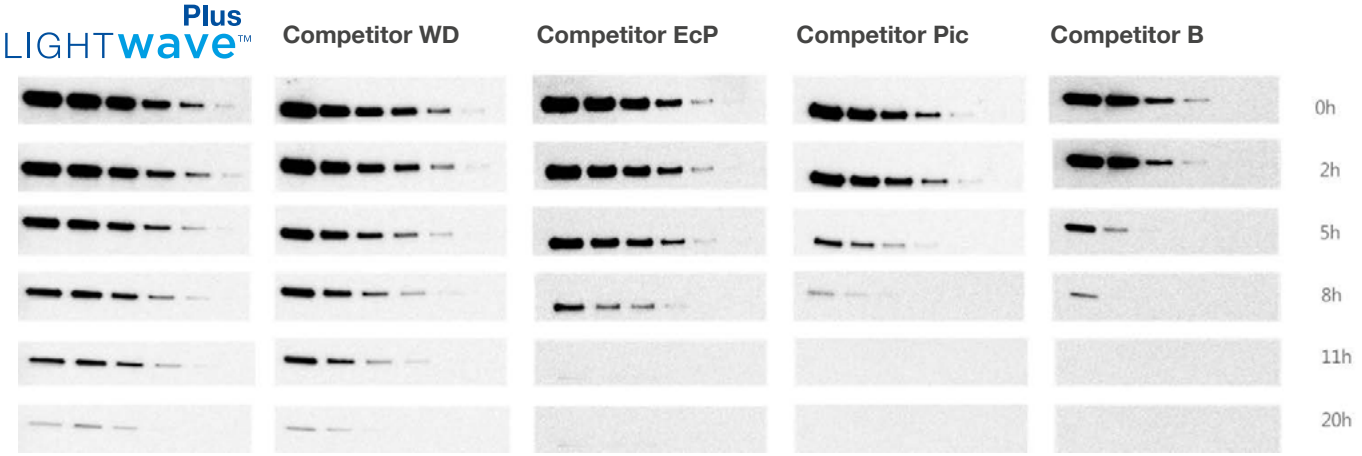
Features

- Mid femtogram detection
- Extended signal duration
- High range flexibility
- Working solution stable for at least three days
- The perfect ECL formulation combining great sensitivity and long signal duration
- Signal duration 25 hours
- Stable for 1 year at RT

GVS LightWave Plus vs Competitor Signal duration

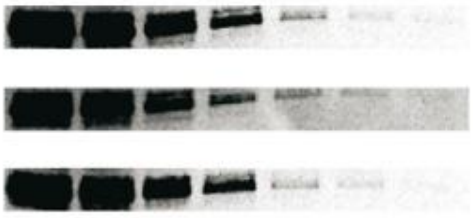
Signal duration

LightWave™ Plus provides an extremely extended signal duration when compared to most mid-level range ECL substrates. The HDAC-1 signal intensity variation over time was analyzed using **LightWave™ Plus** and its competitors (Figure 3).



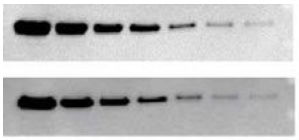
Code	Desciption
LW0003	LightWave™ Plus Western Blotting Substrate 10 ml
LW0004	LightWave™ Plus Western Blotting Substrate 250 ml

GVS Lightwave Max



Competitor F

Competitor E-S



Competitor F

Figure 2. Low background for high sensitive detection with LightWave™ Max.

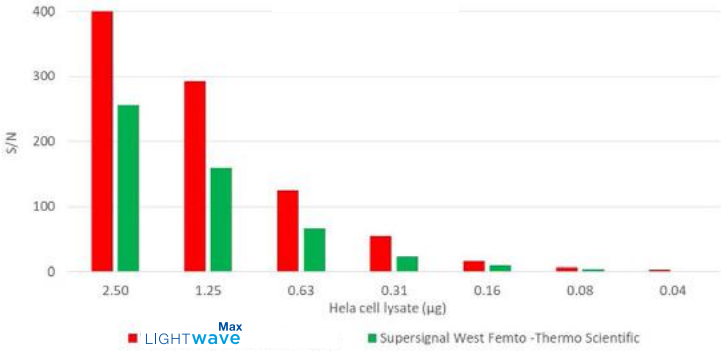
A) Western blotting detection of HDAC-1 on HeLa cell lysate with LightWave™ Max compared to Competitor F. Triplicate blots for each substrate containing 2-fold dilutions of HeLa whole cell lysate were incubated with primary antibody (Rabbit-anti Human HDAC-1) 1:15000 and secondary antibody (Goat anti Rabbit-HRP) 1: 300000 and were simultaneously imaged for 120 seconds with ImageQuant™ LAS 4000 (GE Healthcare).

B) Signal-to-noise ratio (S/N) analysis. LightWave™ Max displays the best combination of sensitivity and signal with low background.

Features

- Low femtogram detection
- Low antibody consumption to save your money
- Working solution stable for at least three days
- The ECL substrate with the highest signal on the market
- Signal duration 8 hours
- Stable for 1 year at RT

Signal to noise ratio



Signal duration

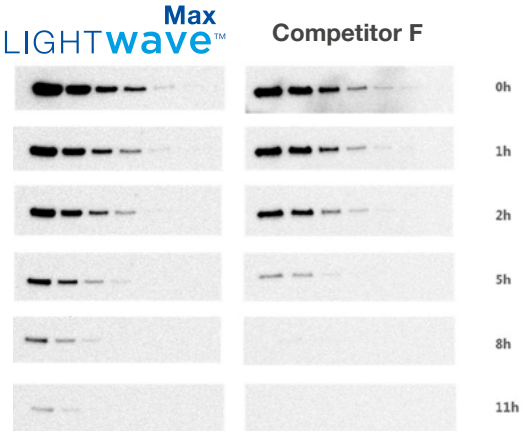


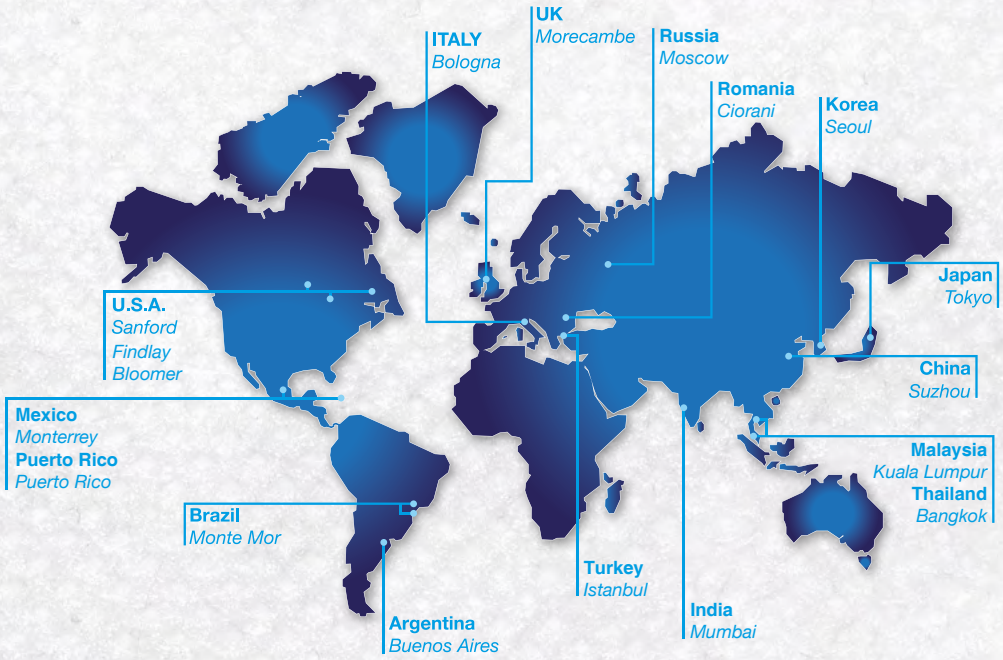
Figure 3. Signal duration of LightWave™ Max compared to Competitor F.
Quadruplicate blots for each substrate containing 2-fold dilutions of HeLa whole cell lysate were incubated with primary antibody (Rabbit-anti Human HDAC-1) 1:15000 and secondary antibody (Goat anti Rabbit-HRP) 1: 300000 and were simultaneously imaged with ImageQuant™ LAS 4000 (GE Healthcare) at time points up to 11 hours post substrate addition.

Code	Desciption
LW0005	LightWave™ Max Western Blotting High Sensitive Substrate 10 ml
LW0006	LightWave™ Max Western Blotting High Sensitive Substrate 100 ml

FILTER TECHNOLOGY

WORLDWIDE

DISTRIBUTION CENTERS



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