

ScanLater Western Blot Assay Kit

Available Kits

Assay Kit	Explorer Kit	Bulk Kit
Eu-Labeled Goat Anti-Mouse ScanLater™ Western Blot Assay Kit	R8201	R8205
Eu-Labeled Goat Anti-Rabbit ScanLater™ Western Blot Assay Kit	R8202	R8204
Eu-Labeled Donkey Anti-Goat ScanLater™ Western Blot Assay Kit	R8224	R8227
Eu-Labeled Donkey Anti-Rat ScanLater™ Western Blot Assay Kit	R8225	R8228
Eu-Labeled Streptavidin ScanLater™ Western Blot Assay Kit	R8200	R8203

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ScanLater Western Blot Assay Kit

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Chapter 1: About the ScanLater Western Blot Assay Kit

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Protein detection is an important task for pharmaceutical and clinical research, and Western Blots (WB), or protein immunoblots, are one of the most common methods to employ for this purpose. Various techniques are used to detect proteins on Western Blot membranes including fluorescence, silver staining, and chemiluminescence. However, each technique has its limitations, and there is a continuous need to improve quantitation, accuracy, and dynamic range of Western Blots.

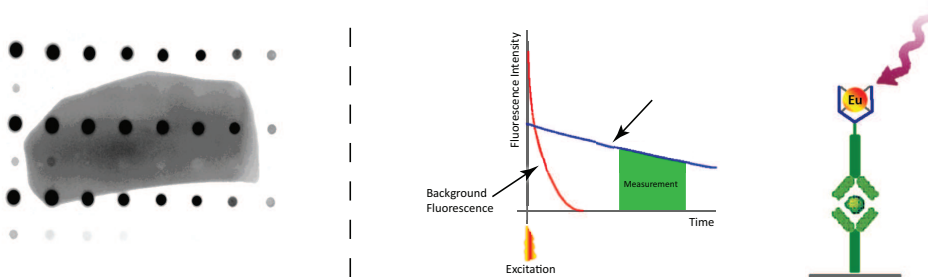
The Molecular Devices ScanLater Western Blot Assay Kit is a novel system for protein analysis you can use with the SpectraMax® iD5 Multi-Mode Microplate reader, SpectraMax® i3x Multi-Mode Microplate Reader, and SpectraMax® Paradigm® Multi-Mode Microplate Reader. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. Europium has a long fluorescence lifetime, on the order of 1 msec, and detection is done in Time Resolved Fluorescence (TRF) mode which significantly reduces background from auto-fluorescence or other sources of short lifetime emissions. Place the membranes into the SpectraMax i3x or SpectraMax Paradigm where they are scanned with the ScanLater® Western Blot (WB) Detection Cartridge or place into the SpectraMax iD5 when equipped with the Enhanced TRF Module.

The method does not involve enzyme detection, and the Eu-chelates are resistant to photo-bleaching, so the signal remains stable for long periods of time (weeks to months). This permits repeat reading of membranes and the potential for comparison of band intensities to known standards for more accurate quantitation. There is also no camera blooming, as can occur with chemiluminescence or fluorescence detection, so the system gives sharp bands and excellent image quality.

The TRF detection employs photon counting, so the theoretical dynamic range is $>10^5$. In practice, however, dynamic range is limited by saturation of binding sites on high-abundant bands and non-specific binding to background membrane.

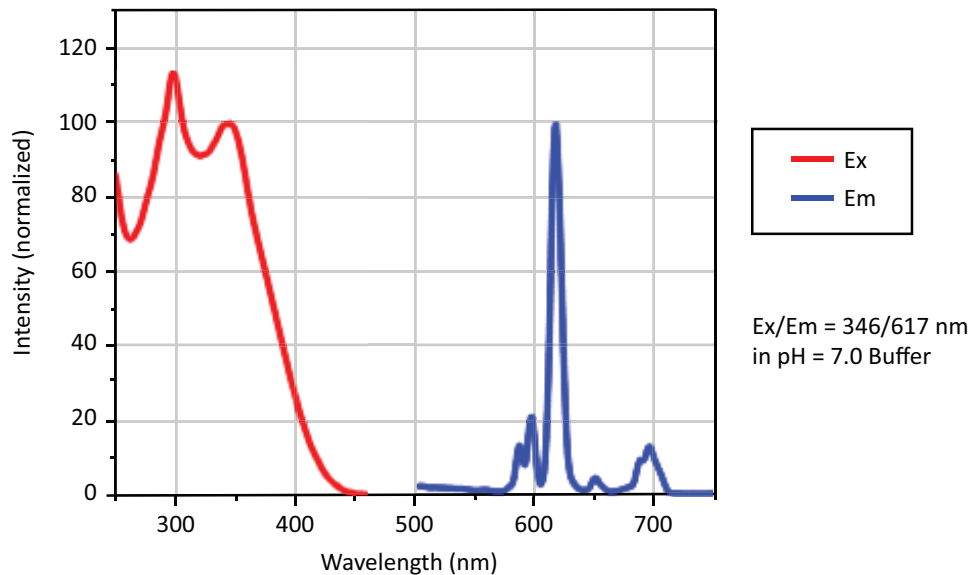
Assay Principle

Europium has a long fluorescence lifetime, on the order of 1 msec, and detection is done in Time Resolved Fluorescence (TRF) mode which significantly reduces background from auto-fluorescence or other sources of short lifetime emissions.



The figure on the left shows a chemiluminescence label with high background due to excess substrate. The figure on the right shows an Eu label with no substrate-concentration issues and less auto-fluorescence.

Excitation and Emission Spectra of Europium



- Excitation maximum 346 nm
- Emission maximum: 617 nm

Advantages

- **Sensitivity:** Detection of low picogram quantities of protein is easy with good primary antisera.
- **Robust workflow:** No substrates needed. Right after washing the secondary antibody, the membrane can be scanned.
- **Stability:** The signal remains stable for long periods of time (weeks to months).
- **Quantitative:** Can generate quantitative results.
- **Reduced background:** Europium has a long fluorescence lifetime, on the order of 1 msec, and detection is done in Time Resolved Fluorescence (TRF) mode which significantly reduces background from auto-fluorescence or other sources of short lifetime emissions.
- **Versatility:** Assay kits are available for rabbit primary antisera, for mouse primary antisera, and for biotinylated tags.
- **Save time:** No time-consuming ECL optimization.
- **Reduce costs:** Eliminate the need for X-ray films and developer.
- **Dynamic range:** The TRF detection employs photon counting, so the theoretical dynamic range is $>10^5$. In practice, however, dynamic range is limited by saturation of binding sites on high-abundant bands and non-specific binding to background membrane.
- **Ready to go:** Works with SpectraMax i3x, SpectraMax iD5, and SpectraMax Paradigm microplate readers.

Applications

- Identify the nature of the protein or epitope effectively. Also, it can be used as a tool for quantitative analysis of protein.
- Use for chromatography components analysis, sucrose gradient analysis.
- Test the endogenous or exogenous expression of phosphoprotein so as to detect the phosphorylation signal.
- Protein resilience in the function experiment.
- Structure domain analysis.
- Analysis of the protein expression level.
- Analysis of protein content in the serum.
- Analysis of regulation protein expressed in the cell cycle.



Kit Components

Components of the ScanLater Western Blot Assay Kits

Item	Explorer Kit	Bulk Kit
ScanLater 10X Washing Buffer	100 mL, 1 bottle	300 mL, 3 bottles at 100 mL each
ScanLater 5X Blocking Buffer	40 mL, 1 bottle	120 mL, 1 bottle
ScanLater Eu-Labeled Antibody or Streptavidin	20 µg, 1 vial	60 µg, 1 vial

Before you use the components of the assay kit, dilute the buffer solutions and reconstitute and aliquot the Eu-labeled antibody or streptavidin. See [Preparing the Reagent on page 12](#).

If you use the Explorer Kit, do not aliquot or freeze the reconstituted antibody or streptavidin. Protect the solution from light and store it at 4°C for up to one (1) week.

The Explorer Kit and the Bulk Kit differ in their dilution requirements.

Dilution Differences in the ScanLater Western Blot Assay Kits

Item	Explorer Kit	Bulk Kit
Reconstitute Eu-labeled antibody or streptavidin	200 µL of 1x blocking buffer	60 µL of reagent-grade water
Recommended dilution of reconstituted antibody or streptavidin for the assay	1:500	1:5000

Components You Can Order Separately

Item	Volume	Part Number
ScanLater 10X Washing Buffer	100 mL	R8206
ScanLater 5X Blocking Buffer	120 mL	R8207
ScanLater Eu-Labeled Goat Anti-Mouse IgG	60 µg	R8208
ScanLater Eu-Labeled Goat Anti-Rabbit IgG	60 µg	R8209
ScanLater Eu-Labeled Donkey Anti-Goat IgG	60 µg	R8226
ScanLater Eu-Labeled Donkey Anti-Rat IgG	60 µg	R8229
ScanLater Eu-Labeled Streptavidin	60 µg	R8212

Materials Required But Not Provided

To use the ScanLater Western Blot Assay Kit, you need the following instrumentation and supplies from Molecular Devices:

- SpectraMax i3x and SpectraMax Paradigm with the ScanLater Western Blot (WB) Detection Cartridge
- SpectraMax iD5 with the Enhanced TRF Module
- ScanLater™ Membrane Holder

Other Required Supplies

Item	Suggested Vendor
Acrylamide gel	No recommendation
Reagent-grade water	No recommendation
Western Blot membranes	For optimal results, use Millipore Immobilon FL (IPFL00010).
Molecular Weight standards	ScanLater Western Blot Protein Ladder Reagent (R8220) from Molecular Devices The ScanLater Western Blot Protein Ladder Reagent contains seven biotinylated proteins in the molecular weight range of 10 kD to 140 kD to be detected using Eu-labeled streptavidin. In addition, three blue-stained protein bands are visibly detected at 18 kD, 31 kD, and 70 kD to monitor migration during gel electrophoresis and to assess the efficiency of protein transfer.
Incubation and wash trays	No recommendation
Primary antibodies	No recommendation
Forceps	No recommendation

Storage and Handling

The ScanLater Western Blot Assay Kits ship at ambient temperature. To ensure product stability, maintain the following storage requirements:

- Store the 10X (or 1X) washing buffer at 4°C.
- Store the 5X (or 1X) blocking buffer at –20°C.
1X blocking buffer can be stored at 4°C for up to three (3) weeks.
- Store the Eu-labeled antibody or streptavidin at –20°C.
- Reconstituted antibody or streptavidin should be aliquoted into smaller volumes and stored at –20°C. See [Preparing the Reagent on page 12](#).

Protect the aliquots from light. Do not freeze and thaw the aliquots more than once. After thawing, the aliquots are stable at 4°C for up to two (2) weeks.

If you use the Explorer Kit, do not aliquot or freeze the reconstituted antibody or streptavidin. Protect the solution from light and store it at 4°C for up to one (1) week.

If stored correctly, the Bulk Kit is warranted for 6 months from the date of shipment, and the Explorer Kit is warranted for 1 month from the date of shipment.



WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: support.moleculardevices.com/

Supported Instruments

The ScanLater Western Blot Assay Kit is designed for use with the following microplate readers:

- SpectraMax i3x with the ScanLater Western Blot (WB) Detection Cartridge
- SpectraMax iD5 with the Enhanced TRF Module
- SpectraMax Paradigm with the ScanLater Western Blot (WB) Detection Cartridge

Prepare the Western Blot using standard blotting procedures for the membrane you use. Let the blot dry for a minimum of 1 hour before detection. You can store dry blots overnight at room temperature on filter paper.



Note: Before you run the protocol, read the entire procedure. It is helpful to also review [Tips and Troubleshooting on page 19](#).

Quick Start Protocol

To run the Quick Start protocol:

1. Dilute the buffer solutions and reconstitute the antibody or streptavidin provided in the assay kit.
2. Transfer the gel to the blotting membrane as required by your application.
3. Soak the membrane in 1X washing buffer for a minimum of 1 minute.
4. Block the membrane in 1X blocking buffer for 1 hour.
5. Add your primary antibody directly into the blocking buffer at the correct dilution for your application and incubate from 90 minutes to 4 hours.
6. Wash the membrane 3 times in 1X wash buffer for 5 minutes each time.
7. Dilute the previously reconstituted antibody or streptavidin in 1X blocking buffer.
 - For the Explorer Kit, the recommended dilution is 1:500.
 - For the Bulk Kit, the recommended dilution is 1:5000.
8. Add the diluted antibody or streptavidin to the membrane and incubate for 60 minutes.
9. Wash the membrane 3 times in 1X wash buffer for 5 minutes each time.
10. Rinse the membrane with water for 15 seconds and let it dry before you scan. You can scan the membrane wet right after the wash step, but a dry membrane has a higher signal compared to its background.
11. Scan the membrane in a SpectraMax i3x, SpectraMax iD5, or SpectraMax Paradigm microplate reader.

Preparing the Reagent

Before you use the components of the assay kit, dilute the buffer solutions and reconstitute and aliquot the antibody or streptavidin.

To prepare the reagent:

1. Dilute the wash buffer to 1X strength by adding 9X its volume of reagent-grade water.
2. Dilute the blocking buffer to 1X strength by adding 4X its volume of the diluted (1X) wash buffer.
3. Spin the antibody or streptavidin vial to move all the lyophilized powder to the bottom of the vial.
4. Reconstitute the antibody or streptavidin in reagent-grade water.
 - For the Explorer Kit, dissolve the antibody or streptavidin in 200 μ L of 1X blocking buffer.
 - For the Bulk Kit, dissolve the antibody or streptavidin in 60 μ L of reagent-grade water.

Allow 30 minutes of gentle shaking for full solubility before use.

5. Aliquot the reconstituted antibody or streptavidin into smaller volumes and store at -20°C . Protect the aliquots from light. Do not freeze and thaw the aliquots more than once. After thawing, the aliquots are stable at 4°C for up to two (2) weeks.

If you use the Explorer Kit, do not aliquot or freeze the reconstituted antibody or streptavidin. Protect the solution from light and store it at 4°C for up to one (1) week.

Preparing the Membrane for the Assay



Note: Handle membranes by their edges only, using clean forceps. Do not touch the membrane with gloved or bare hands.

To prepare the membrane:

1. Transfer the gel to the blotting membrane as required by your application.
2. Soak the membrane in 1X washing buffer for a minimum of 1 minute.
3. Place the membrane in an incubation tray and block using 1X blocking buffer for 1 hour with gentle shaking. Use enough blocking buffer to completely cover the membrane.
4. Add your primary antibody directly into the blocking buffer at the correct dilution for your application and mix well.
5. Incubate the blot in the diluted primary antibody from 90 minutes to 4 hours at room temperature with gentle shaking or overnight at 4°C.
6. Wash the membrane by pouring off the primary antibody solution, rinsing with 1X wash buffer, covering the blot with 1X wash buffer, and then shaking it on a platform shaker for 5 minutes at room temperature. Pour off the wash solution and repeat this wash process 2 more times.
7. Dilute the previously reconstituted antibody or streptavidin in 1X blocking buffer. Prepare enough volume to completely cover the membrane.
 - For the Explorer Kit, the recommended dilution is 1:500.
 - For the Bulk Kit, the recommended dilution is 1:5000.Optimize the dilution for your application.
8. Add the diluted antibody or streptavidin to the membrane after the washing is complete.
9. Incubate for 60 minutes at room temperature with gentle shaking.
10. Wash the membrane by pouring off the secondary antibody solution, rinsing with 1X wash buffer, covering the blot with 1X wash buffer, and then shaking it on a platform shaker for 5 minutes at room temperature. Pour off the wash solution and repeat this wash process 2 more times.
11. Rinse the membrane with water for 15 seconds and let it dry before scanning. The membrane can be scanned wet right after the wash step, but a dry membrane has a higher signal compared to its background.

Loading the Membrane into the Membrane Holder



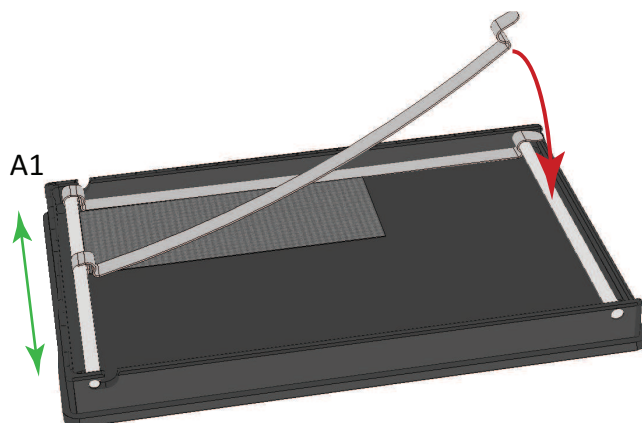
Note: Handle membranes by their edges only, using clean forceps. Do not touch the membrane with gloved or bare hands.

To scan a membrane, you must first place it in a Molecular Devices ScanLater membrane holder.

The maximum size of a membrane that will fit in the membrane holder is 109 mm x 77 mm.

To load the membrane into the membrane holder:

1. Place the membrane on the membrane holder with one corner of the membrane aligned with the A1 corner of the membrane holder.



2. Lower the holder clips into place over the membrane as close to the edges of the membrane as possible to expose the maximum scanning area of the blot.
3. Gently press the end of each holder clip to snap it into place and secure the membrane to the holder.



CAUTION! To prevent damage to the instrument or the membrane holder, make sure that all the holder clips are securely snapped into place before you insert the membrane holder into the instrument.

4. Load the membrane holder into the plate drawer with the A1 corner in the A1 position of the drawer. See the instrument user guide.

Running the Western Blot Assay

Before you scan the membrane, define the settings for the SpectraMax i3x, SpectraMax iD5, or SpectraMax Paradigm using a SoftMax® Pro Software protocol.

The following table lists the recommended experiment setup parameters.

Experiment Setup Parameters

Parameter	Setting
Excitation wavelength (fixed)	340/80 nm
Emission wavelength (fixed)	616/10 nm
Read area	Mini Membrane
Default scan resolution	Low
ROI scan resolution	High
Attenuation (SpectraMax Paradigm only)	Off

After you load the membrane into the membrane holder, load the membrane holder into the instrument plate drawer with the A1 corner of the holder in the A1 position. See [Loading the Membrane into the Membrane Holder on page 14](#).

The maximum size of a membrane that will fit in the membrane holder is 109 mm x 77 mm.

A high resolution scan of the entire surface can take approximately 15 minutes. Define a smaller area for the scan to reduce the scan time.

Molecular Devices strongly recommends that you optimize the parameters for your application.

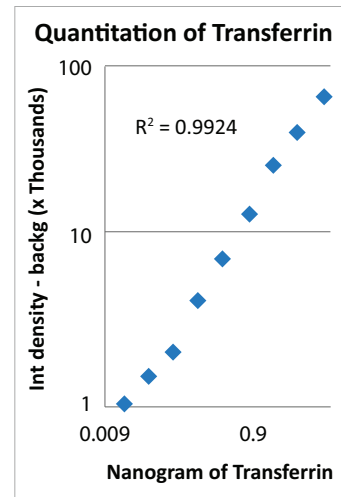
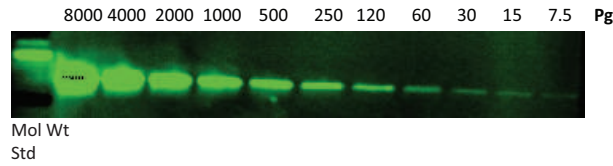
After scanning a membrane for Western Blot data, the data are displayed in the SoftMax Pro Software as an image. You can use the image tools in the Plate section to zoom, crop, colorize, and adjust the intensity of the image. You can also select a region of interest (ROI) and rescan the membrane at a higher resolution.

Western Blot membrane data are saved as a TIFF image that can be analyzed by the image-analysis tool of your choice. The SoftMax Pro Software comes with an installed version of the ImageJ software from U.S. National Institute of Health (NIH).

For more information, see the SoftMax Pro Software application help or user guide.

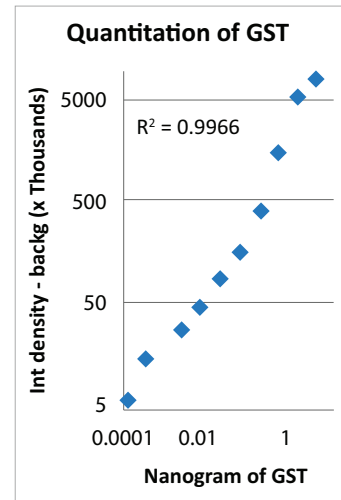
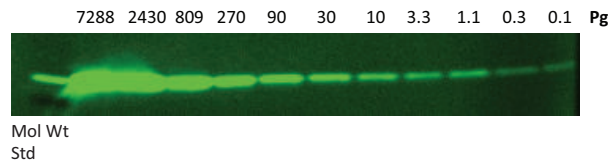


Western Blot Scan of Transferrin



In this assay, a serial two-fold dilution of transferrin was loaded on the gel starting with 8 ng. Proteins were transferred from the gel to Immobilon FI. The membrane was blocked for 1 hour with 1X blocking buffer, followed by probing with rabbit anti-transferrin. The secondary antibody used was Eu-labeled anti-rabbit antibody. Blots were dried and scanned on a SpectraMax Paradigm Multi-Mode Microplate Reader using a ScanLater Western Blot (WB) Detection Cartridge.

Western Blot Scan of GST



In this assay, a serial dilution of GST was loaded on the gel starting with 50 ng. Proteins were transferred from the gel to Immobilon FI. The membrane was blocked for 1 hour with 1X blocking buffer, followed by probing with biotin-labeled rabbit anti-GST. Eu-labeled streptavidin was used for detection. Blots were dried and scanned on a SpectraMax Paradigm Multi-Mode Microplate Reader using a ScanLater Western Blot (WB) Detection Cartridge.



Tips and Recommendations

- Follow the protocol carefully.
No single-blocking reagent is optimal for every antigen-antibody pair. Some primary antibodies can exhibit greatly reduced signal or different nonspecific binding in different blocking solutions. If you have difficulty detecting your target protein, changing the blocking solution can dramatically improve the performance. If the primary antibody has worked well in the past using chemiluminescent detection, try that same blocking solution for detection. Other commonly used blocking buffers other than BSA are 3% casein and 5% non-fat milk.
- To prevent background speckles on blots, use high-quality, ultra-pure water for buffers. Rinsing previously-used incubation boxes with methanol can reduce background contamination of future blots.
- Never do Western Blot incubations or washes in dishes that have been used for Coomassie staining.
- Handle membranes by their edges only, using clean forceps. Do not touch the membrane with gloved or bare hands.
- Always pour off antibody solution and washes from the same corner of the box to ensure complete removal of previous solutions.
- After handling membranes that have been incubating in antibody solutions, clean the forceps thoroughly with distilled water or methanol, and then rinse with distilled water.
- Always clean the membrane holder before loading a new membrane for scanning to remove dust, buffer residue, and smudges that can have a negative effect on image quality or contaminate the membrane. The scanning surface can be wiped with methanol, rinsed with a small volume of water, followed by a final rinse with isopropanol to help prevent blot contamination. Pressurized “canned air” can be helpful in removing dust and lint.
- Do not wrap the membrane in plastic when scanning.
- Store the secondary antibody vials at -20°C in the dark. Do not thaw and refreeze antibodies, as this will have an effect on the performance. Minimize exposure to light and take care not to introduce contamination into the vial. Dilute immediately before use. If particulates are seen in the antibody solution, centrifuge before use.
- Use gels that contain the narrowest well size possible to minimize load volume and concentrate the target protein.
- The best transfer conditions, membrane, and blocking agent for each experiment vary, depending on the antigen, sample type, and antibody.
- For proteins <100 kDa, the recommended transfer buffer is 1X Tris-glycine buffer with 20% methanol and no SDS. Addition of SDS to the transfer buffer can greatly reduce binding of transferred proteins to the membrane (for both PVDF and nitrocellulose).
- For proteins >100 kDa, decrease the methanol concentration in the transfer buffer to 10%.
- Do not over-block. Extended blocking times can cause loss of target protein from the membrane.
- Do not do an overnight wash with washing buffer.

Troubleshooting

Problem: High background, uniformly distributed

- Compare different blocking buffers to find the most effective for your system; try blocking longer.
- Try diluting blocker 1:1 in 1 X washing buffer
- **Background on nitrocellulose**
Increase the quantity of Tween 20 added to the diluted antibodies, staying in the range of 0.05% to 0.2%. Add SDS to diluted secondary antibody, staying in the range of 0.01% to 0.02%.
- **Background on PVDF**
Reduce Tween 20 in diluted antibodies to 0.05%. Add 0.01% to 0.02% SDS to diluted secondary antibody.
- **Antibody concentrations too high**
Optimize primary and secondary antibody dilutions.
- **Insufficient washing**
Increase number of washes and buffer volume. Make sure that 0.05% Tween 20 is present in the wash buffer and increase if needed. Note that excess Tween 20 (0.5% to 1%) can decrease signal.
- **Cross-reactivity of antibody with contaminants in blocking buffer**
Use Blocking Buffer instead of milk. Milk-based blockers can contain IgG that can crossreact with anti-goat antibodies. This can significantly increase background and reduce antibody titer. Milk-based blockers can also contain endogenous biotin or phosphoepitopes that can cause higher background.
- **Insufficient antibody volume used**
Use enough antibody volume so that the entire membrane surface is sufficiently covered with liquid at all times. Use heat-seal bags if the volume is limiting.
Do not let an area of the membrane dry out. Use agitation for all antibody incubations.
- **Membrane contamination**
Always handle membranes carefully and with clean forceps.
Do not let the membrane dry.
Use clean dishes, bags, or trays for incubations.

Problem: Uneven, blotchy or speckled background

- **Blocking multiple membranes together in small volume**
If multiple membranes are being blocked in the same dish, ensure that the blocker volume is sufficient for all membranes to move freely and make contact with liquid.
- **Membrane not fully wetted or permitted to partially dry**
Keep membrane completely wet at all times. This is particularly crucial if the blot will be stripped and re-used.
If using PVDF, remember to first pre-wet in 100% methanol.
- **Contaminated forceps, dishes, or transfer equipment**
Always carefully clean forceps after they are dipped into an antibody solution, particularly a dye-labeled secondary antibody. Dirty forceps can deposit dye on the membrane that will not wash away.
Use clean dishes, bags, or trays for incubations

- **Dirty membrane tray**
Clean the membrane tray carefully before each use. Dust, lint, and residue will cause speckles.
- **Incompatible marker or pen used to mark membrane**
Use only pencil to mark membranes.

Problem: Weak or no signal

- Do not do an overnight wash with washing buffer.
- **Not using optimal blocking reagent**
Primary antibody might do substantially better with a different blocker.
Try diluting the blocker 1:1 in 1X washing buffer.
- **Insufficient quantity of antibody used**
Primary antibody might be of low affinity.
Increase the quantity of antibody or try a different source.
Extend primary antibody incubation time. For example, try 4 to 8 hours at room temperature, or overnight at 4°C.
Increase the quantity of primary or secondary antibody, optimizing for the best performance.
- **Primary or secondary antibody might have lost reactivity due to age or storage conditions**
Use new or unexpired antibodies.
- **Too much detergent present; signal being washed away**
Decrease Tween 20 or SDS in diluted antibodies. Recommended SDS concentration is 0.01% to 0.02%, but some antibodies can require an even lower concentration.
- **Insufficient antigen loaded**
Load more protein on the gel. Use the narrowest possible well size to concentrate antigen.
- **Protein did not transfer well**
Check transfer buffer choice and blotting procedure.
Use pre-stained molecular weight marker to monitor transfer, and stain gel after transfer to make sure proteins are not retained in gel.
- **Protein lost from membrane during incubations**
Reduce blocking times or decrease high concentrations of detergent in diluted antibodies.
- **Proteins not retained on membrane during transfer**
Addition of 20% methanol to transfer buffer can improve antigen binding.



Note: Methanol decreases pore size of gel and can hamper the transfer of large proteins.

SDS in the transfer buffer can interfere with binding of transferred proteins, especially for low molecular weight proteins. Try reducing or eliminating SDS.



Note: The presence of up to 0.05% SDS improves the transfer efficiency of some proteins.

Small proteins can pass through the membrane during transfer (“blow-through”). To prevent this, use a membrane with a smaller pore size or reduce the transfer time.

Problem: Nonspecific or unexpected bands

- **Antibody concentrations too high**
 - Reduce the quantity of antibody used.
 - Reduce antibody incubation times.
 - Increase Tween 20 in diluted antibodies.
 - Add or increase SDS in diluted secondary antibodies.
 - Increase salt in wash buffer up to 500 mM of salt.
- **Not using optimal blocking reagent**
 - The choice of blocker can have an effect on background bands. Try a different blocker.

Obtaining Support

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Our Support website—support.moleculardevices.com/—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Please have the instrument serial number (on the rear of the instrument), or reagent kit lot number, and any related sample data files available when you call.

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