Resolve to Do Better Western Blotting

BIO-RAD

Introducing the *new* GelDoc Go Imaging System.

Meet the GelDoc Go. In just three clicks, you can simplify your gel documentation by easily capturing nucleic acid gels, protein gels, or colorimetric blots. Although it is small in size, it can image large gels with the sensitivity and performance you expect from Bio-Rad. You can even excise your bands right on the system. It's truly ready, set, and GelDoc Go.

See why this is the GelDoc of your dreams.
bio-rad.com/GelDocGo

#ScienceForward

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Welcome

The western blotting or protein blotting process was originally developed in 1979. This process has become a powerful and popular technique for the visualization and identification of proteins. The western blotting process is continually being improved and optimized for more accurate, sensitive, quantitative, and rapid results. This book will bring you up to speed on all the new advances such as stain-free technology, total protein normalization, 3 minute rapid transfer technique, 5 minute rapid universal blocking, and state-of-the-art advances in digital imaging.

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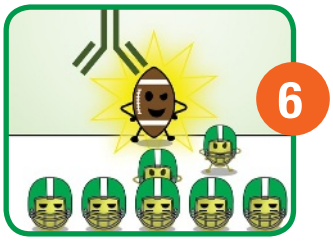
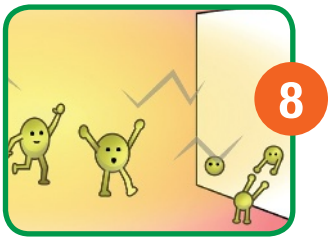
Resolved: Tips for Better Western Blotting

Time to do it right!
Save valuable time on optimizing protein transfer with a rapid blotting transfer system.



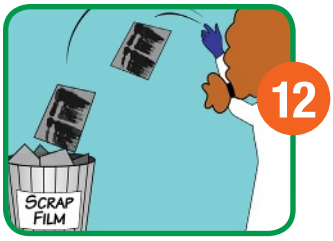
Blockheaded!
Take the guesswork out with a five-minute blocking buffer for ALL western blots.



Where's my protein?
Verify your protein transfer with Stain-Free Imaging.

Saturation or publication?
Normalize your loading controls to generate quantitative, publication-ready data.

Meet the Innovators: Bio-Rad Digital Imaging Systems



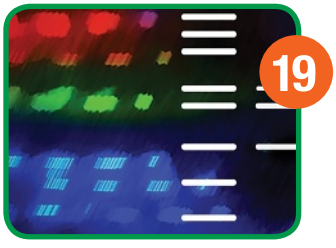
Get out of the darkroom!
Advanced digital imaging systems achieve the sensitivity of film without the disadvantages.

Enabling the customer to do everything they can do with film, and more.

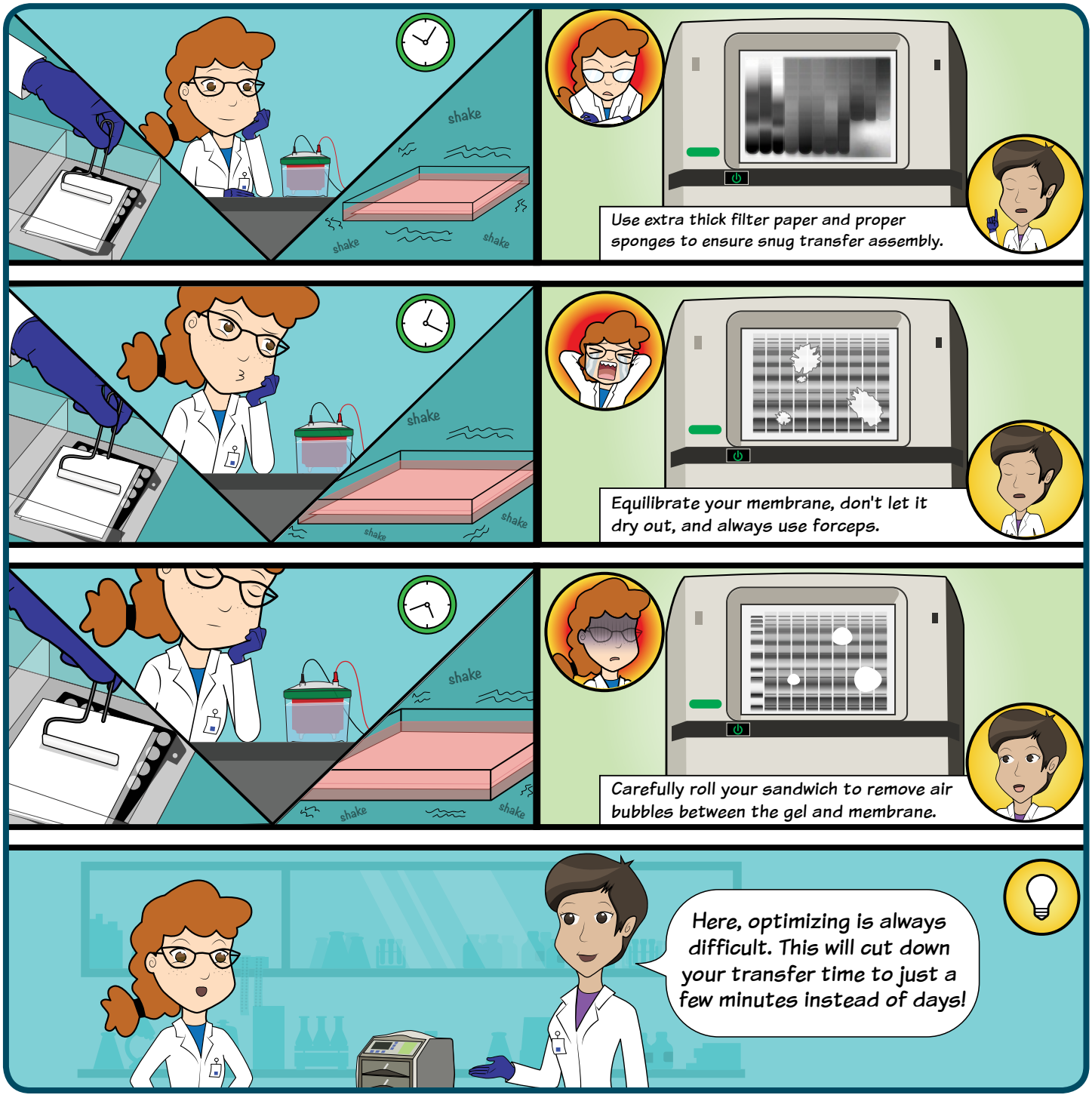
A multidisciplinary group effort, very focused on customer needs.

ChemiDoc MP: trying to make the best imaging system in the world.

Webinar Series: Mastering the Art and Science of Western Blotting



Resolved: “Groundhog Day”



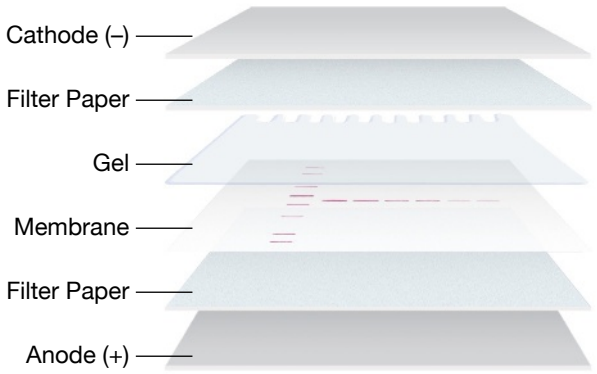
Time to do it right!
If you didn’t have the time to get it right the first time, when will you find time to redo it?
A rapid blotting transfer system enables you to quickly optimize your transfer conditions.

Rapid Transfer Systems

Types of Blotting Systems

There are three types of transfer systems:

1. Traditional wet tank transfer systems—gel and membrane are submerged under transfer buffer in tank
2. Semi-dry transfer systems—gel and membrane are sandwiched between buffer-wetted filter paper in direct contact with electrodes
3. Rapid transfer systems—use specialized materials and buffers optimized for rapid and efficient protein transfer



Gel and Membrane Setup for Electrophoretic Transfer

Pros and Cons of Wet Tank, Semi-Dry, and Rapid Blotting Systems

Tank systems can be used for transfers of proteins of all sizes; they allow for a flexible range of power and time settings. On the downside, they require some manual assembly and typically require cooling. Transfer times are long—30 minutes to overnight.

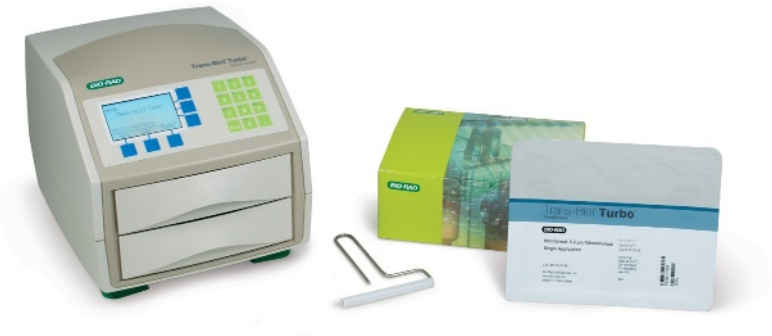
Semi-dry systems are typically easier to set up than tank systems and can complete transfer in 15–60 minutes. They don't require cooling, but power and transfer time options are limited. They perform best for proteins in the range of 30–120 kD.

Rapid transfer systems are a more recent development. These systems utilize specialized buffers and filter papers and come pre-wetted and sealed in single-use packages, simplifying assembly of the transfer stack. They can be used to transfer proteins over a broad size range and don't require cooling. Rapid transfer systems are very efficient and can complete transfer in only 3–10 minutes.

The Trans-Blot Turbo Rapid Blotting Transfer System

The Trans-Blot Turbo Transfer System is a high-performance western blotting transfer system designed to provide rapid transfers with high efficiency. The system enables blot transfer of protein in as little as 3 minutes, with performance comparable to traditional tank transfer.

Trans-Blot Turbo Transfer Packs include an optimized buffer, membrane, and filter paper combination that provides superior blot transfers.



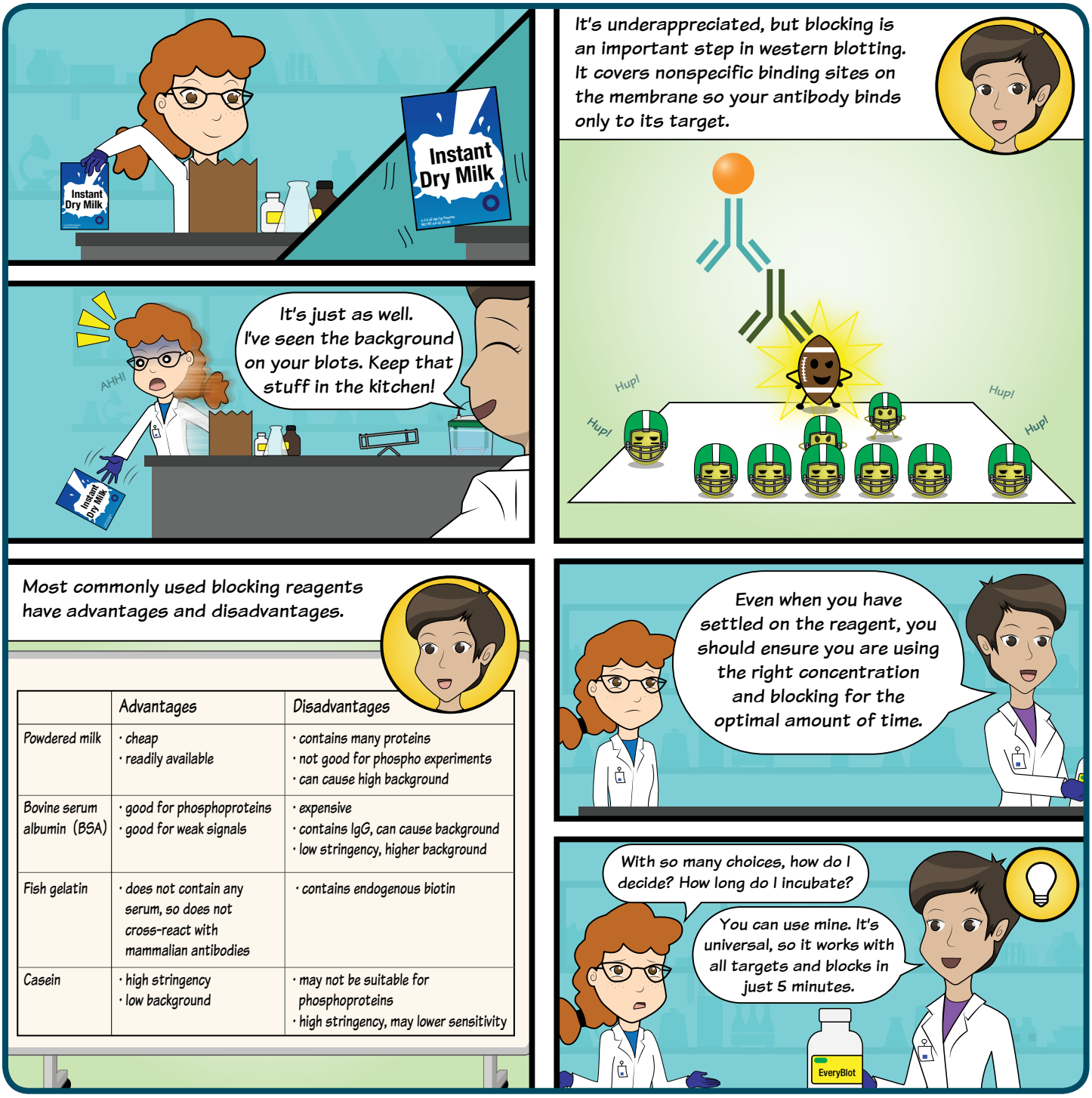
bio-rad.com/TransBlot

Learn More

Six Tips for Efficient Protein Transfer

bio-rad.com/6TransferTips

Resolved: “Gridiron”



	Advantages	Disadvantages
Powdered milk	· cheap · readily available	· contains many proteins · not good for phospho experiments · can cause high background
Bovine serum albumin (BSA)	· good for phosphoproteins · good for weak signals	· expensive · contains IgG, can cause background · low stringency, higher background
Fish gelatin	· does not contain any serum, so does not cross-react with mammalian antibodies	· contains endogenous biotin
Casein	· high stringency · low background	· may not be suitable for phosphoproteins · high stringency, may lower sensitivity

Blockheaded!
Not sure you’re using the best blocking buffer for your blot and antibodies? Take the guesswork out of the process with a five-minute blocking buffer for ALL western blots: EveryBlot Blocking Buffer.

Blocking Reagents

Following transfer, before immunodetection, unoccupied antibody binding sites on the membrane must be blocked to prevent nonspecific antibody binding. Failure to completely block these sites can lead to high backgrounds that obscure the signal.

The best blocker for each experiment will depend on the antibody and membrane type. Optimize the detection system for maximal signal with lowest background by testing several blocking agents. Modern formulations like Bio-Rad's EveryBlot Blocking Buffer are universal, performing well across a wide range of targets, sample types, and detection methods.

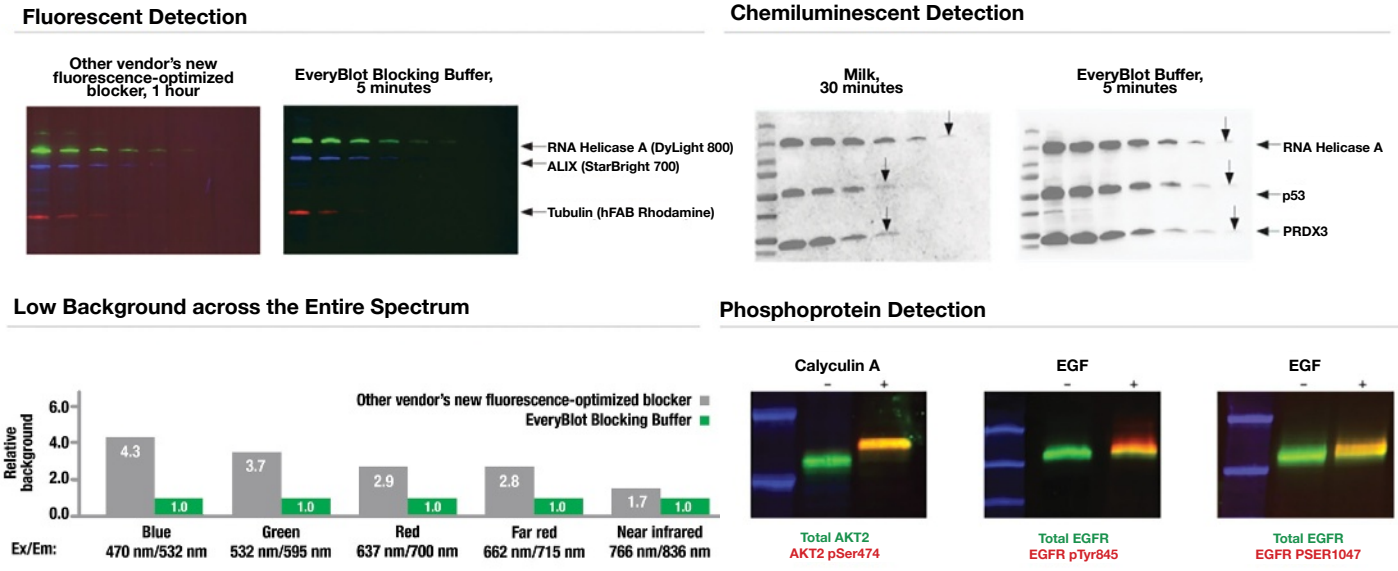
EveryBlot Universal Blocking Buffer

EveryBlot Blocking Buffer provides 5 minute blocking and maximum sensitivity for all western blots regardless of detection method.

- Reduces nonspecific antibody binding to reduce background while maintaining excellent sensitivity
- Compatible with both chemiluminescent and fluorescent detection
- Contains no phosphate-based buffers. Ideal for use with phospho-specific antibodies
- Complete blocking in 5 minutes

See the Data

EveryBlot Universal Blocking Buffer outperforms other blocking buffers in all western blotting applications:

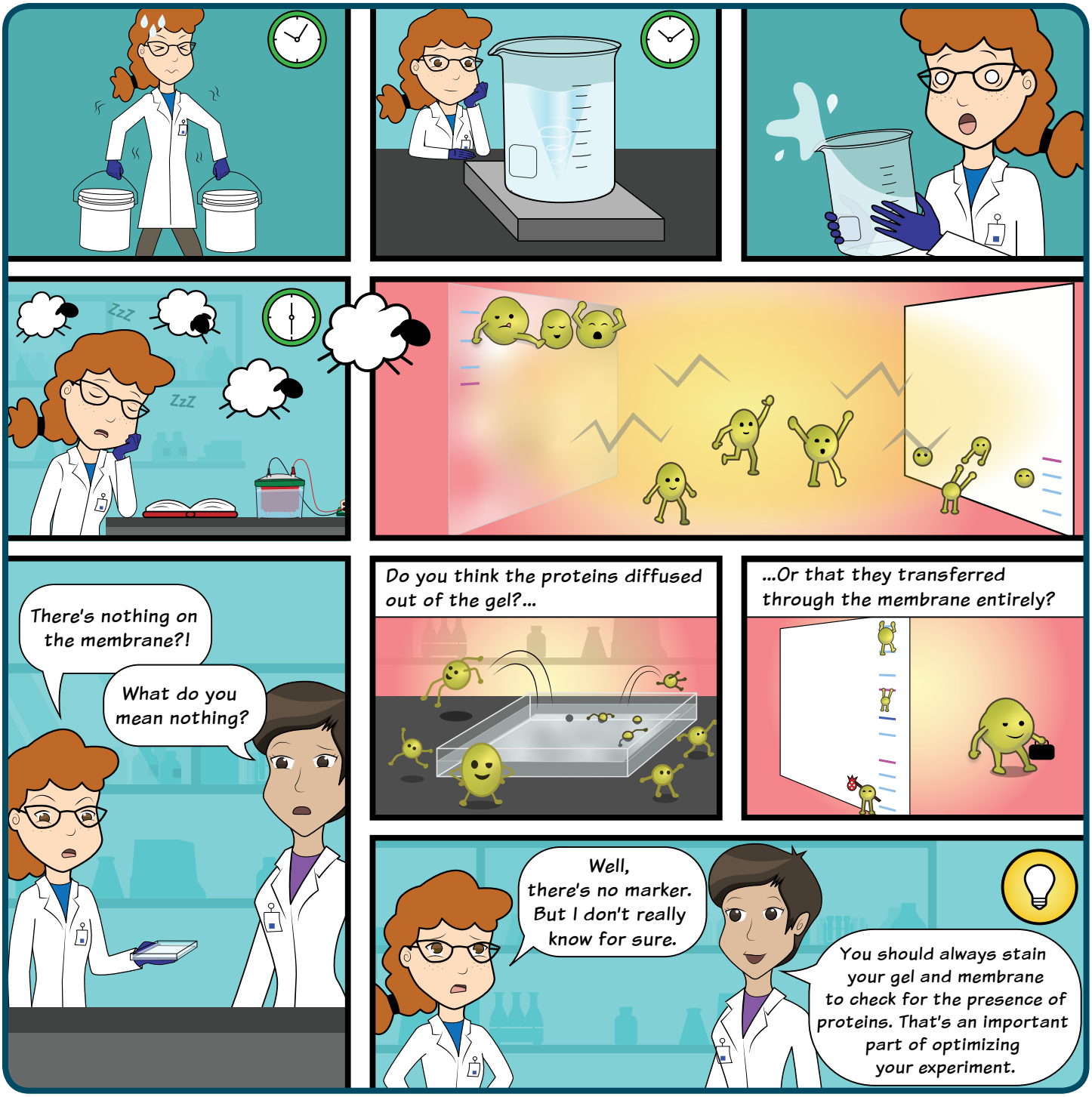


Learn More

EveryBlot 5 Minute Universal Blocking Buffer

bio-rad.com/EveryBlot

Resolved: “Stuck in the Matrix”



Where’s my protein?
You need to know what’s going on with the proteins both in your gel and on your blot. But how? Stain-free gel and blot imaging.

Stain-Free Gel and Blot Imaging

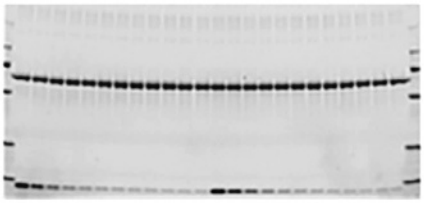
Validating Protein Transfer

One way to determine if the proteins in your gel were completely transferred to the membrane is by total protein staining. Unfortunately, the use of total protein stains can interfere with subsequent blot development and visualization steps, typically requiring tedious staining and destaining procedures.

Stain-Free Imaging

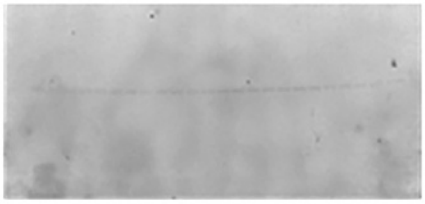
Stain-Free Imaging technology uses a proprietary trihalo compound that is cast into the acrylamide gel that covalently binds to tryptophan residues in the sample upon brief UV activation. After transfer, images of the gel or membrane can easily be captured multiple times without staining and destaining steps. This allows visualization, verification, and validation at all steps of electrophoresis and blotting, saving time wasted on western blots with problems that would not otherwise have been detected until the later stages of blot processing and development.

Verification of Western Blotting Protein Transfer Using Stain-Free Imaging



Gel before Transfer

Membrane after Transfer



Gel after Transfer

Assessment of protein transfer using a Stain-Free enabled imaging system. Images of the gel before and after transfer and of the membrane after transfer were taken using a stain-free enabled imager. Serial 1:2 dilutions of hemoglobin (starting quantity, 80 ng), with 1.8 µg of BSA/lane as a carrier (top band), were electrophoretically separated on a 4–20% 26-well Criterion Stain-Free gel.

Stain-Free Western Workflow

Bio-Rad's Stain-Free Western Workflow streamlines your western blotting protocol, providing greater speed and validation at each step of a western blotting experiment—from running gels to quantitating proteins.

The complete Stain-Free Western Workflow incorporates innovative tools including our proprietary stain-free gels and the ChemiDoc MP Imaging System.

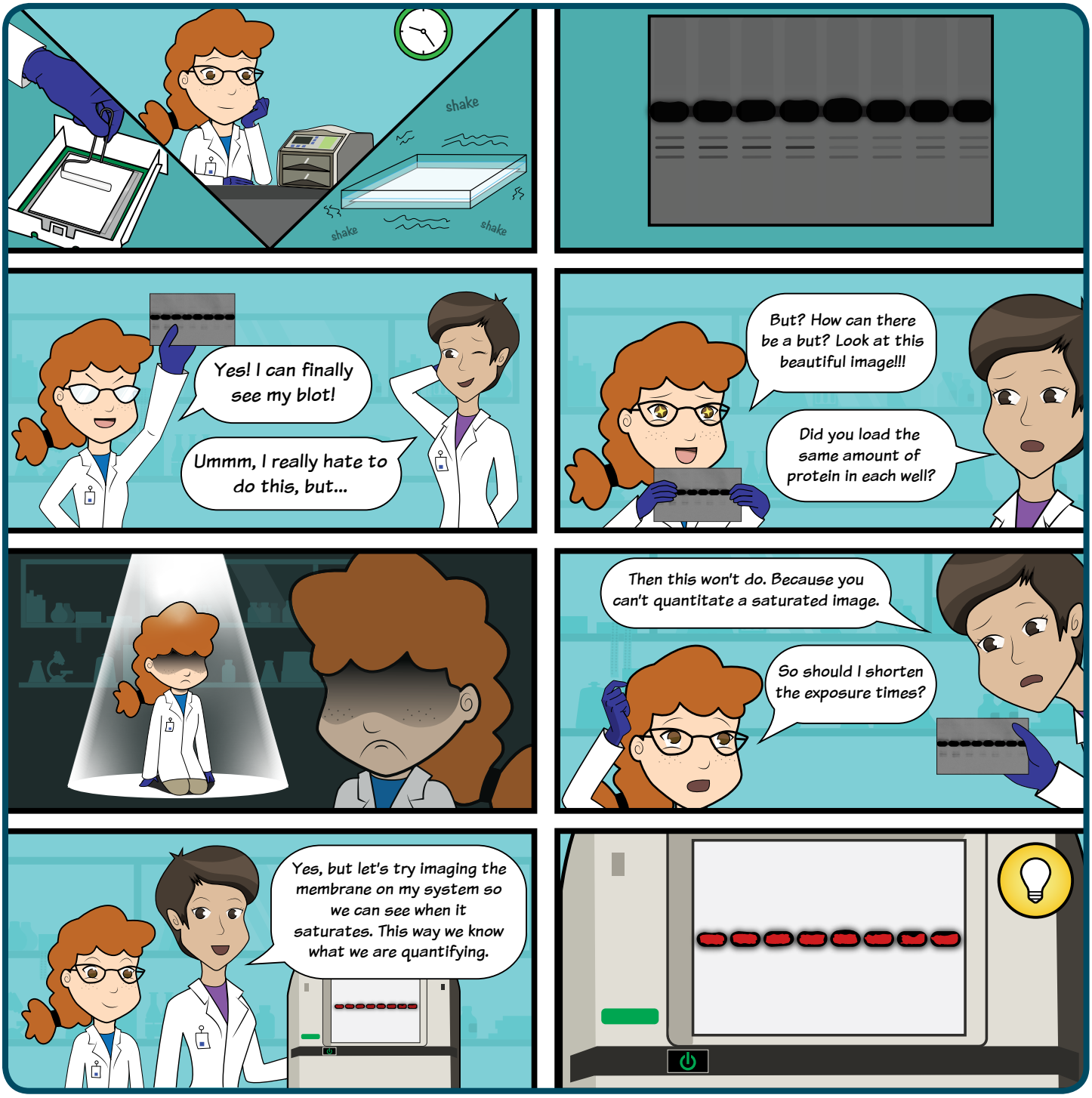
bio-rad.com/WesternWorkflow

Learn More

Stain-Free Imaging Technology ▶

bio-rad.com/StainFree

Resolved: “Almost Doesn’t Count”



Saturation or publication?
Normalization of your loading controls is the key to obtaining quantitative results. Learn how total protein normalization using a stain-free imaging system can make the difference between quantitative blots you can trust and blots that may not be publishable.

Total Protein Normalization

Blot Normalization Methods

Western blot normalization enables you to accurately compare changes in target protein expression by correcting for inconsistencies in sample preparation, pipetting, and protein transfer. Normalization is required to establish a correlation between changes in band intensities and biological changes in your samples.

Conventionally, a "housekeeping protein" (HKP) such as β-actin, β-tubulin, or GAPDH is used as a loading control, with the assumption that the expression levels of these proteins remains constant.

Problems with Housekeeping Proteins

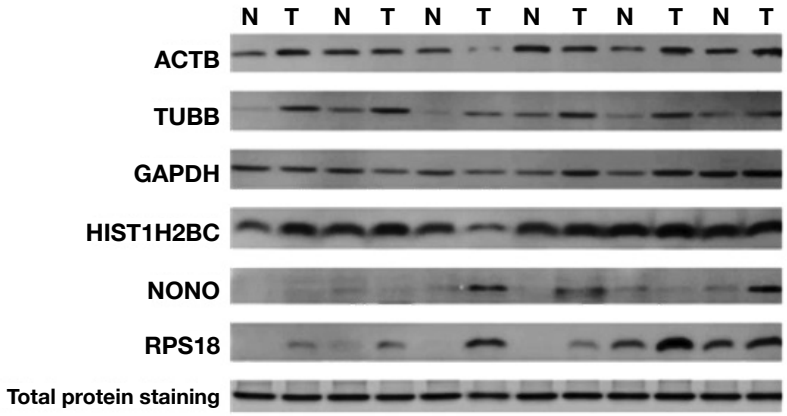
The use of HKPs for western blot normalization is now known to be problematic.[1] Reliable assessment of changes in target protein expression levels requires measurement of both the target protein and the loading control protein in their linear dynamic ranges for immunodetection. Unfortunately, housekeeping proteins are usually highly expressed, whereas target proteins are often expressed in low abundance. Thus, to detect the target protein of interest, large amounts of cell lysate may need to be loaded. This may result in overloading of HKPs, yielding oversaturated reference bands, out of their linear range. Furthermore, HKP expression levels may not be constant, but instead vary with different experimental treatments and other factors.

[1]Gomes A V. Can We Trust Western Blots? Paper presented at: Experimental Biology; April, 2014; San Diego, CA.

Variation in Housekeeping Protein Expression Levels

The expression level of housekeeping proteins can change due to:

- Experimental conditions
- Developmental changes
- Post-transcriptional regulation
- Differences in cell tissue age and type



Differences in five candidate housekeeping proteins and total protein staining between tumor and noncancerous tissues in the validation sample set. Immunodetection measurements of housekeeping protein levels show poor linearity and do not accurately indicate cell lysate loading levels. Total protein normalization of HKPs yields consistent band intensities. (Hu X, Du S, Yu J, Common housekeeping proteins are upregulated in colorectal adenocarcinoma and hepatocellular carcinoma, making the total protein a better "housekeeper." Oncotarget 2016; 7(41): 66679–66688).

Stain-Free Imaging for Total Protein Normalization

The use of total protein measurement for western blot loading controls (total protein normalization; TPN) is a method devised to eliminate the risk that expression of the loading control is affected by experimental conditions. Total protein levels can be determined by staining the membrane with total protein stains; however, as discussed previously, these present their own difficulties.

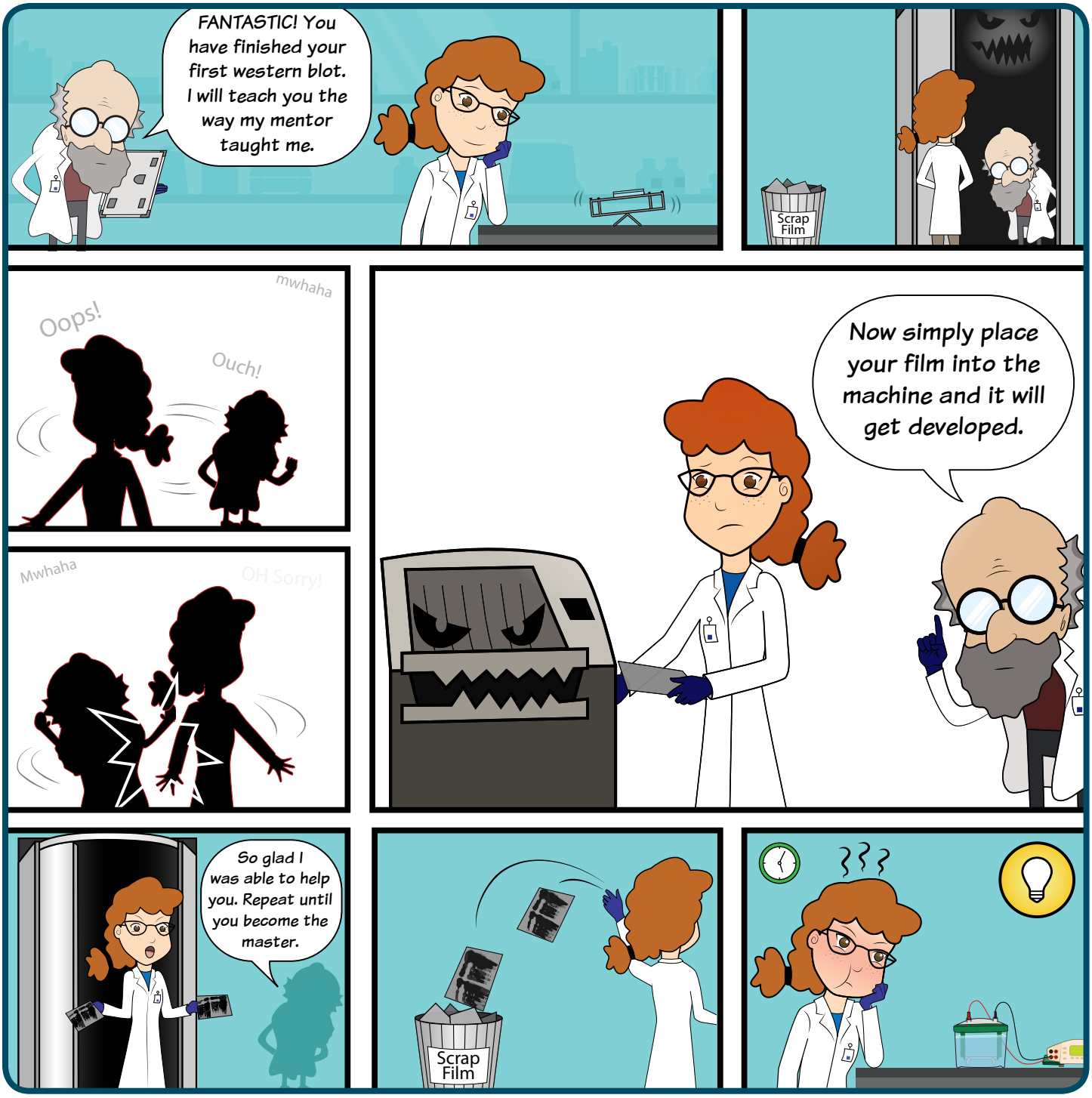
Stain-Free Imaging with TPN has thus emerged as the best method for quantitative western blotting.

Learn More

Total Protein Normalization ▶

bio-rad.com/TPN

Resolved: “The Gremlin”



Time to get out of the darkroom!
Advances in digital imaging systems provide you the sensitivity of film, or greater, without the hassle, expense, and waste disposal problems of film.

Film vs. Digital Imaging for Western Blotting

Advantages of Digital Imaging Systems

Photographic film has long been used for radiolabeled and visible light imaging in southern and western blotting, with one of its main virtues being sensitivity. Almost any signal can be captured with a sufficiently long exposure. Film use, however, comes with significant drawbacks: the time and cost of film development and waste disposal, the limited dynamic range of film imaging, and difficulty of acquiring quantitative data using film.

Digital imaging systems eliminate these disadvantages and deliver very fast results with simpler imaging techniques. Furthermore, integrated software makes data acquisition and analysis much easier, particularly quantitation. With the digital revolution in consumer photography, advanced digital imaging systems for the lab have greatly improved. Digital sensitivity now equals or exceeds film, and the broad dynamic range enabled by digital imaging systems allows for accurate quantitation of a range of signal intensities, along with other advantages over film.

Key Benefits of Digital Imaging Systems:

- Wide dynamic range ≥ 4 logs
- Quantitative
- Digital documentation
- Environmentally friendly—no chemicals!
- No costly consumables (film)
- Initial capital investment typically recovered in as little as 1 year

Digital Imaging for Quantitative Western Blotting

The reliable quantitation of digital imaging systems makes them the best choice for a quantitative blot assay. Normalizing blots with housekeeping proteins (HKPs) presents problems with HKP expression variability and measurement linearity, especially in the mismatch between typically highly expressed HKPs and target proteins. Total protein normalization using Stain-Free Imaging allows normalization over a wide dynamic range.

As the integrity of quantitative blot data has been increasingly called into question, journals have begun revising their publication standards for this data. The use of X-ray film has been specifically cited for its low dynamic range, leading to saturation problems. A stain-free enabled digital imaging system enables you to consistently get publication-quality data.

New Publication Requirements

"Housekeeping proteins should not be used for normalization without evidence that experimental manipulations do not affect their expression."

Revised guidelines for authors from

Journal of Biological Chemistry

jbcresources.asbmb.org/collecting-and-presenting-data

Learn More

Advantages of Digital Imaging Over Film ▶

bio-rad.com/DigitalWestern

ChemiDoc MP Imaging System: Imagine No Compromises

With the ChemiDoc MP System, the superior quantitation of digital meets the sensitivity of film and delivers the best fluorescence performance with the brightest new fluorophore.

Move Beyond Traditional Imaging

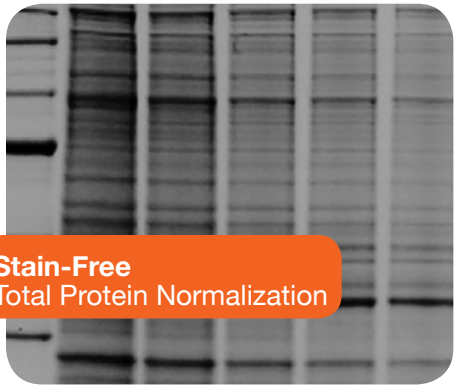
Traditional laser scanning systems and X-ray film are a thing of the past. Get higher multiplexing capabilities without sacrificing the performance you expect from a best-in-class imaging system.

Download a Brochure or Request a Demo

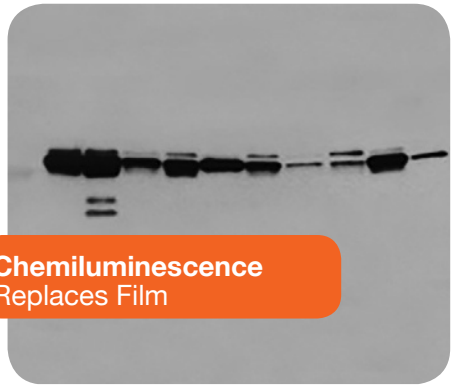
bio-rad.com/ChemiDocMP

More Options, More Data

With the Chemidoc MP, one imaging system covers all of your gel documentation, chemiluminescence, and multiplex fluorescence needs. (The stain-free channel is used in addition to the other channels, but cannot be used at the same time.)



Stain-Free
Total Protein Normalization



Chemiluminescence
Replaces Film

Multiplex Fluorescence
Three Colors

Better Experience

Image Lab Touch Software

Image Lab Touch Software works with the ChemiDoc MP Imaging System and provides easy and intuitive onboard acquisition software. Built for labs with multiple users, Image Lab Touch Software teaches as you tap and explore, selects optimal light source, and permits both manual entry and automated calculation of exposure times.

Learn More

bio-rad.com/ImageLab

Meet the Innovators:
Inside Bio-Rad's Digital Imaging Lab

Steven Swihart

What is your role at Bio-Rad?
I'm the Advanced Engineering Manager in the Life Science Group. We work on developing things from the initial ideas to the point that they mostly work and can be handed off to engineering.

How did you explore the problem of increasing sensitivity?
Sensitivity is a lot of things, all driven by signal to noise. In my mind, the most relevant explanation is the ability to see a really rarefied signal plenty clearly above any background. There's many ways to approach increasing sensitivity. You could use a faster lens or a larger sensor. You could have a sensor with better dark current or lower read noise. So, we looked at several different ways to approach the problem.

What were some challenges in reducing background noise?
For quantitation purposes, it's completely irrelevant what the source of the noise is; whether it's from the sample itself, or if it's from anything inside of the system, like an electronic source of noise, or just a diffuse scatter, from some surface somewhere, or something glowing. Doesn't matter. It all ends up being noise that can be seen in the customer's picture, so we needed to identify and address all the sources.

Are there particular noise challenges with different imaging modes?
We had to ask ourselves a number of questions in developing the fluorescence capabilities. They all really came down to this: If you put the sample down anywhere on the imaging surface, do you get the same signal to background for any given band?

Steven Swihart, MSME/MSAE,
Advanced Engineering Manager,
Life Science Research, Bio-Rad Laboratories

"When you set out to beat film with a digital imager, you have to allow the customer to do all the things they can do with film—and more."

Are there any other image artifacts that have appeared regardless of the source? We had to try all of the spectral channels with a whole series of different stains to make sure that there was nothing untoward happening, and that the customer can place a sample anywhere on the tray and a given band will give the same intensity.

Can you give us an example of a specific noise problem you tackled and the solution you came up with?
As we were working our way through the noise problems in the fluorescence channels, we

Meet the Innovators...

eventually came to a floor that we could not get through. We realized that we needed to improve the black coatings in the instrument in several places, and the coating on the glass elements of the lens. All of the paints that we had tried had different characteristics, but none of them really cut it. So, we finally ended up with a unique black coating that we applied to all of the exposed metal surfaces inside the imager. The lens elements required a specific black paint to be applied to their edges, and we had a custom multilayer broadband AR coating applied to all the glass elements to increase transmission, especially in the IR.

What were some other challenges in increasing sensitivity, for example, in low-light performance?

Imaging sensitivity is really important in light-starved applications like chemiluminescence western blotting, typically far-red or NIR fluorescence applications. The reason that our optical system is so good at taking pictures in low light is that it's flat-out optimized for it. It has a ridiculously low dark current CCD. It has a very fast lens that's optimized for just the spectral ranges that we're operating in. It has a zoom stage that you can maximize for the smallest sample. You can take a picture zoomed in to just a small sample to use the entire image sensor for just that area, or you can take a picture of multiple blots at the same time if that's your choice. We also use custom, high-power LEDs for fluorescent illumination.

The sum of all the customization and design that we did for the camera itself, lenses, filtration, and illumination ended up being unique enough in terms of its performance capabilities that we wrapped it all up into a patent. ●

Evan Thrush

Evan Thrush, PhD, Principal Optical Engineer, Life Science Research, Bio-Rad Laboratories

What is your role at Bio-Rad?

I'm a Principal Optical Engineer in the Life Science Group, working in the area of optics, and sensing, and fluorescence. I'm a physicist, and I always wanted to do something applying physics to life sciences, and optical sensing bridges that gap. Optics involves a lot of physics, and in life science, there's a lot of optical measurements being done. I'm impressed that it's a very multidisciplinary group here. I work closely with the application-side people—biologists and chemists—with experts working in these areas for 15–20 years, and everybody gets involved from day one of the project.

What were some specific goals of the ChemiDoc MP project?

We wanted to match the performance of laser scanners and also be able to do chemiluminescent imaging and gel imaging. We considered using lasers, but we ended up using custom LEDs and custom filters. If you filter an LED properly, it behaves a lot like a laser, and LEDs are very reliable

Meet the Innovators...

sources—extremely stable. Lasers can suffer from speckle problems. But the biggest advantage of LEDs is the sheer amount of power you can get from these devices. When we started the project, we didn't know if we could achieve the performance of a laser scanner, but by the end of the project, everyone was very happy with the performance comparisons.

Were there other special challenges in developing this multifunctional imaging system?
With a camera-based imaging system, filtering is challenging. You have light coming in from all different angles, you have this big image sensor that you have to protect from all this stray light. Our first-generation product, the older ChemiDoc, used only one layer of filtering, just a filter in front of the lens. For this new instrument, we developed what I view as four different levels of filtering. We have filters behind the lens, we have a filter in front of the lens, and lens itself acts as a spatial filter, which filters out all the highly off-angle light and parasitic light. So we have a lot more sophisticated filtering in this new product, and it really is what enabled us to get good performance in the near-infrared in particular.

How did you source and select the camera and filter components?
One of the unique requirements for the camera in this system was our filter positioning. You cannot just go and get a commercial off-the-shelf camera that is capable of doing the things we need to do for our imaging system. Our requirements were complex enough that we needed to have a custom lens developed for us.

What benefits did custom camera development bring?
One of the nice things about going custom was that we could make the resolution a bit higher than what we could find from off-the-shelf lenses. We could also optimize the lens coatings—antireflection coatings—which is important to get the most light through the lens. We did some specialty coatings that you wouldn't find in a standard lens; we coated the inside of the lens with special black materials that mitigate some of the parasitic stray light that can bounce within lenses. It basically helps reduce the optical background levels that you see. ●

Kevin McDonald

Kevin McDonald, PhD, Senior Staff Scientist, Life Science Research, Bio-Rad Laboratories

"There was always one goal with the ChemiDoc MP imaging project: to make the best imaging system in the world."

What is your role at Bio-Rad?
I'm a cell biologist by training. I've been at Bio-Rad working on imaging projects since 2001. In developing imaging systems, we really have to know what the customer needs are, so I try to keep my finger on the pulse of all the new

Meet the Innovators...

applications out there. We're in a unique position to know the customer's needs because we've been doing Western blotting for a very long time.

"Multiplexing is very important for an imaging system, and for western blotting specifically, because it makes your life much more efficient."

What drove the development of the new ChemiDoc MP?
Our legacy instrumentation was wanting in a few spaces. Sensitivity was pretty good, but we still wanted to do better. Resolution was perceived as insufficient, and multiplexing capabilities were really starting to be demanded by our customers. So, we had to put all those together in a single package, which led us to go to a completely new platform, with a completely new camera, in order to fulfill all these needs.

What are the advantages of multiplexing?
Multiplexing is very important for an imaging system, and for western blotting specifically, because it makes your life much more efficient. You can put multiple antibodies on one membrane, which saves you money and time. It allows you to do things you couldn't do with separate blots. It's difficult to judge how much relative sample you have from one blot to another. If you have everything on one membrane, you can compare expression levels of one antigen to another, and you can do phosphorylation studies.

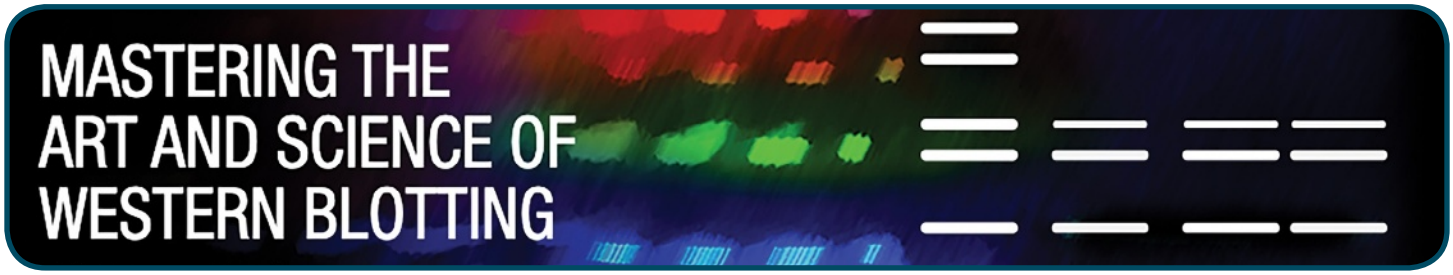
What motivates you in your work?
As a scientist, data integrity is extremely important to me. I design instruments as if I'm going to be the one using them. Bio-Rad has been very adamant about the quality of the data forever in its imaging instruments. We have patents on image correction for blot and gel sampling from the 90's. So, we have quite a track record of working on these problems and how to solve them. And we have more patents this century, regarding how to calibrate and improve imaging quality.

Do you feel you accomplished your goals in the ChemiDoc MP project?
I consider the ChemiDoc MP to be the best system out there for a number of reasons. One: It's breadth of capabilities. This thing can do your nucleic acid samples, it can do your acrylamide samples, and it can do your western blotting samples. We're not really restricting a customer to do what our system can do. We have a system that allows the customer to do what they want to do.

What other features of the instrument are you particularly proud of?
We spent a lot of time with the user interface. This is really important. I can make the greatest, most powerful, whiz-bang thing in the world, but if somebody can't use it, it has no value. If we can't make a piece of equipment that enables a scientist to go out and get data easily, quickly, and accurately, then we failed. It's very, very important that we enable scientists to get their job done. I think that this system is the best option out there for multiplex imaging that a customer can get, if they want to enable themselves to do their science.

See the Full Video Interviews ▶
bio-rad.com/ImageQuality

Webinar Series



Understand the factors that are crucial to successful western blotting

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