ODYSSEY® CLx

QUANTITATIVE WESTERN BLOTS

HIGH SENSITIVITY

WIDE, LINEAR DYNAMIC RANGE

NO FILM OR DARKROOM

WIDE RANGE OF APPLICATIONS

LI-COR®

Experience Excellence

ODYSSEY® CLx Infrared Imaging System

Quantitative Western Blots

The accuracy and linearity of infrared fluorescent detection provides confidence in differences of protein expression

High Sensitivity

Infrared laser technology offers the best detection at the optimum wavelengths

Wide, Linear Dynamic Range

A broad, linear dynamic range accurately detects both strong and weak bands on the same blot, without the uncertainty and inconvenience of multiple exposures

No Film or Darkroom

Save valuable time and money on film and darkroom expenses. Eliminate "blown out" lanes and the need for multiple exposures

Wide Range of Applications

Infrared Western Blots, In-Cell Western™ Assays, On-Cell Western Assays, Coomassie-Stained Gels, DNA Gels, Protein Arrays, EMSAs, Tissue Section Imaging, Whole Organ Imaging, Small Animal Imaging, ELISAs

The Standard for Western Blot Technology

As a researcher, your goal is to efficiently present the most accurate data possible. For more than 30 years, traditional chemiluminescent detection with film has provided data that have been published by scientists worldwide.

Over the past decade, LI-COR® has provided infrared (IR) fluorescent technology with optimized reagents for IR laser-based instrumentation to revolutionize methods for protein detection. This technology has become the standard for quantitative Western blots and eliminated the need for film. Traditional chemiluminescent detection with film provides proven sensitivity, and LI-COR offers industry-leading technology to maintain that sensitivity and improve your data quality to make it the clearest and most accurate it can be. LI-COR has used its expertise in optical design to provide methods for both chemiluminescent, as well as infrared fluorescence protein detection, without the use of film.

Benefits of Digital Imaging with the Odyssey CLx:

- Save valuable money on film and substrates (Table 1)
- Eliminate costs related to darkroom maintenance
- No need for multiple exposures
- A wide, linear dynamic range without saturation or "blown out" lanes
- Accurate detection of strong and weak bands in one exposure
- Sensitivity equal to or greater than that of film
- Eliminate the need for excessive washes and hazardous waste associated with film development
- Reduce the negative environmental impact related to film development. For more information, please visit: *www.licor.com/green*

Whether you are looking to improve your Western blot data by simply moving to digital chemiluminescent detection or by transitioning to quantitative Western blot technology using infrared fluorescent imaging, we will help you find the best solution for you and your lab.

The Infrared Fluorescence Advantage

Advancing Western Blots with Infrared Fluorescence Detection

LI-COR® pioneered infrared Western blots more than ten years ago. Using infrared detection offers numerous benefits, when used with corresponding infrared fluorescent-labeled secondary antibodies.

- Quantitative analysis and a wide linear dynamic range that is not available with traditional chemiluminescent methods
- Detect strong and weak bands on the same blot, without blowouts or hidden bands (Fig. 1)
- Detect two targets simultaneously on the same membrane to increase quantification accuracy
- At the 700 nm and 800 nm infrared wavelengths, both autofluorescence and light scatter are dramatically reduced (Fig. 4)
- Infrared dyes offer advanced signal stability that allows for convenient and reproducible data that are not time-sensitive – data are not contingent on the lifespan of an enzymatic reaction
- More than 4,000 peer-reviewed publications cite data from Odyssey® Imaging Systems

The dynamic nature of enzyme labels and film allows you to capture only a snapshot of the enzymatic reaction and is highly dependent on timing and exposure, limiting linear range and offering only partially quantitative results (Fig. 2). With the infrared fluorescence method, film and enzyme labels are replaced with infrared fluorescent-labeled antibodies.

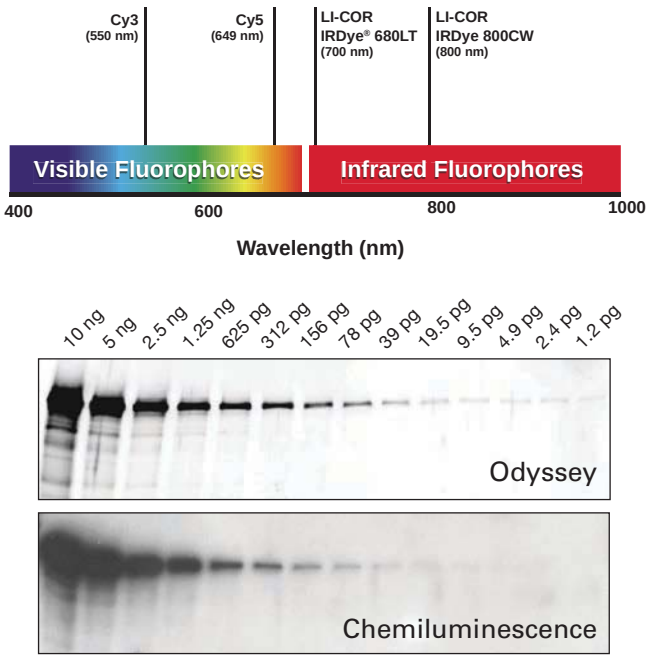


Figure 1. Serial dilutions (10 ng to < 1 pg) of purified human transferrin (Tf) were used to assess Western blot sensitivity. An example of typical results obtained with Odyssey imaging technology. The above data illustrate the detection of 1.2 pg of Tf, while only 4.9-9.5 pg is detected with chemiluminescence. **Infrared fluorescent detection sensitivity is approximately 200-fold greater than other studies with visible fluorophores (Cy®3, Cy®5, or FITC).** (Data generated on Odyssey Classic)

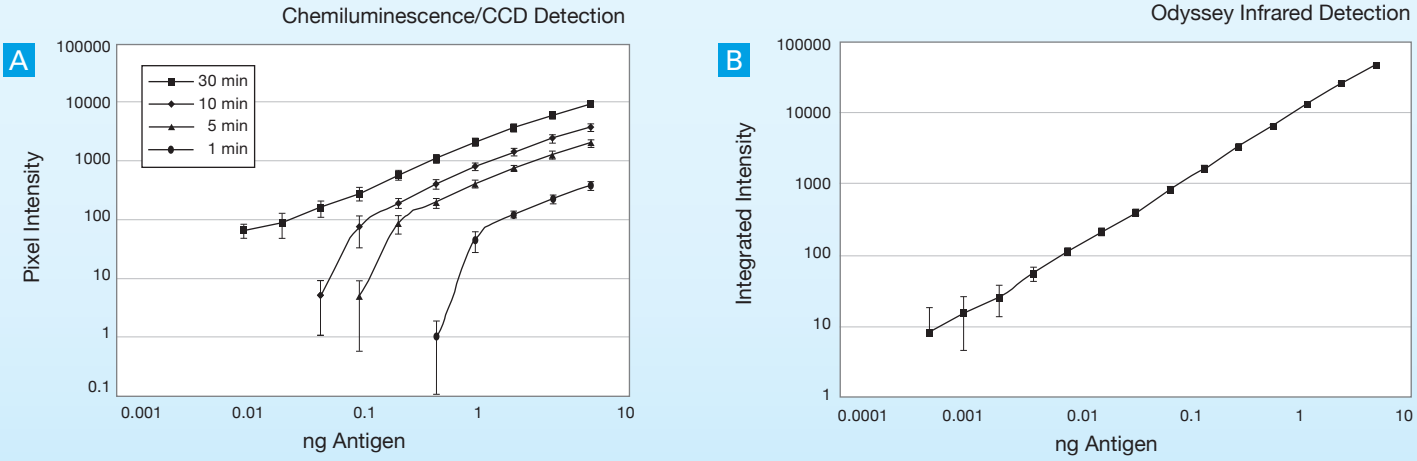


Figure 2. A dot blot assay was used to compare the linear ranges of chemiluminescent and infrared fluorescent detection. Dilutions of mouse antibody were spotted and detected with HRP- or IRDye® infrared dye-labeled goat anti-mouse antibodies. Chemiluminescent data (Panel A) were collected using ECL substrate and a CCD camera with varying exposure times; the infrared image (Panel B) was obtained in a single scan with Odyssey infrared imaging technology. For a 30-minute chemiluminescent exposure, the data set was linear over a 250-fold dynamic range, but not proportional. By contrast, **infrared detection displayed a quantitative linear range greater than 4000-fold (3.6 orders of magnitude)**. A paper detailing this study can be downloaded at *www.licor.com/chemiccd*. (Data generated on Odyssey Classic)

Sensitivity

The Odyssey CLx platform uses infrared laser excitation that out-performs LED and visible white light systems (Fig. 3) for Western blots. Biological materials, membranes, and plastics produce high background due to light scattering and autofluorescence in the visible wavelength range used by most fluorescent imagers. This limits the sensitivity of visible fluorescent systems and makes it difficult to detect low-abundance proteins at endogenous levels without saturation of stronger bands. Infrared laser excitation results in the highest signal-to-noise ratios, and the best detection sensitivity available with a fluorescent system.

Infrared lasers offer high-speed detection with increased sensitivity when compared to systems that use LED and visible white light. This increased sensitivity offers a clear image of your data that is unmatched by other digital imagig systems.

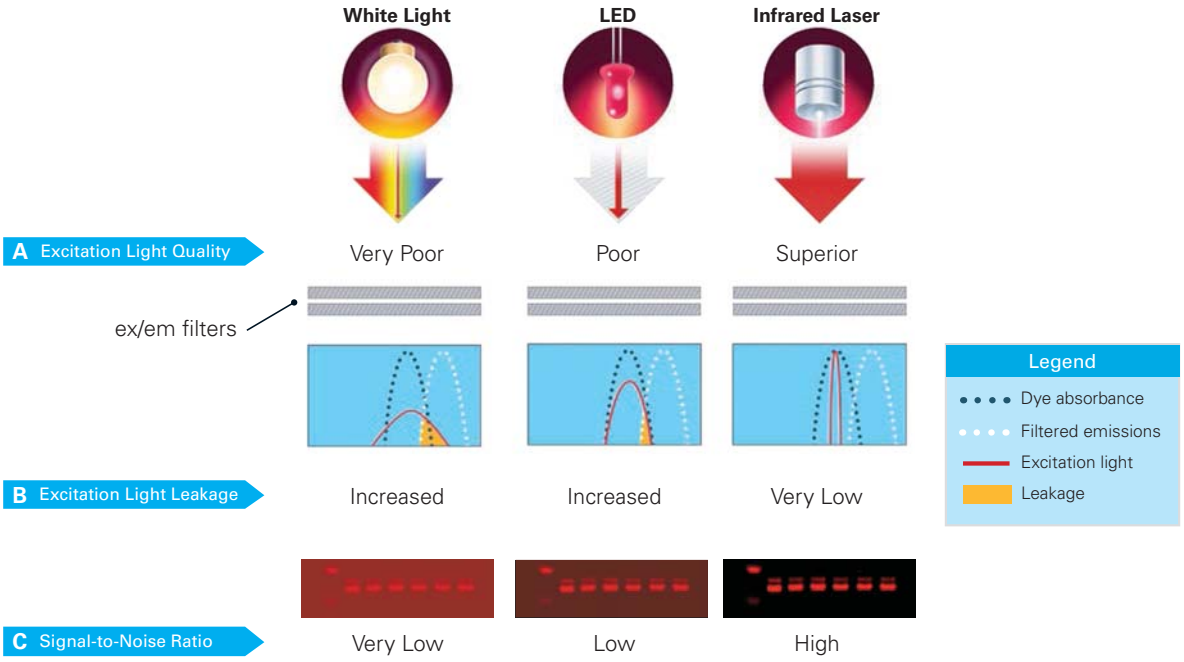


Figure 3. Improved performance using infrared lasers. A) Infrared lasers deliver excitation light in the narrow wavelength band desired, unlike LED or white light sources. B) Leakage of excitation light (yellow shading) increases image background. C) LI-COR filtering technology dramatically reduces excitation light leakage, for decreased image background and sensitive detection of low abundant targets.

LI-COR's laser technology has the best detection at the optimum wavelengths. **When compared to other instruments using infrared fluorescence, the 700 nm and 800 nm channels within the Odyssey family of imagers are always the most sensitive.**

Membrane Autofluorescence is Dramatically Reduced

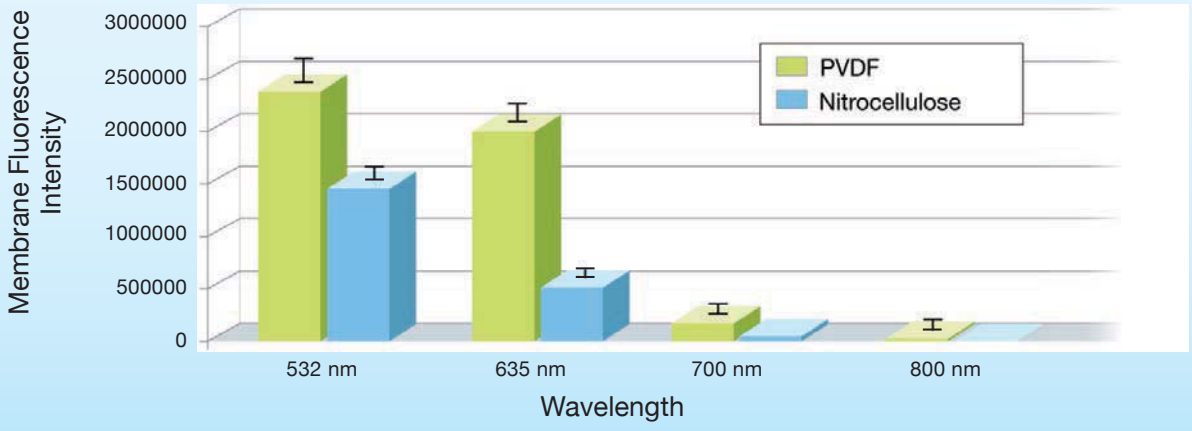


Figure 4. Nitrocellulose and PVDF membranes were imaged with Odyssey infrared imaging technology at Intensity = 5 for both 700 nm and 800 nm wavelengths. The same membranes were scanned at a 532 nm and 635 nm wavelength with a PMT = 500 on a GenePix® 4100A (Molecular Devices). Autofluorescence was much lower at infrared wavelengths.

Wide Dynamic Range

Through the innovative use of infrared fluorescent antibody conjugates, Odyssey imaging systems provide a broad, linear dynamic range to accurately detect both strong and weak bands on the same Western blot. By contrast, the dynamic, enzymatic nature of chemiluminescence allows you to capture only a "snapshot" of the enzymatic reaction and is highly dependent on timing and exposure, limiting linear range and offering only qualitative or partially quantitative results.

Multiplex Detection

The Odyssey Family of Imagers provides simultaneous two-color target analysis with the 700 nm and 800 nm infrared fluorescent detection channels (Fig. 5). Two-color Western blot analysis makes normalization easy by using one channel for normalizing. It also eliminates errors introduced by stripping and reprobing or by comparison of separate blots. Superior image clarity and detail make it easier to detect subtle mobility shifts caused by protein modifications such as phosphorylation.

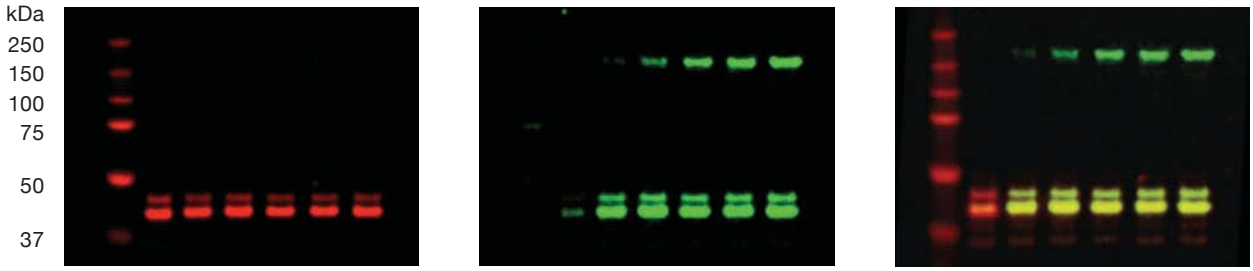


Figure 5. Detect two targets and monitor protein phosphorylation. Lysates (10 µg/well) of A431 cells treated with EGF were separated and transferred to nitrocellulose. The blot was probed with rabbit anti-ERK1 and mouse anti-phospho-ERK primary antibodies (Santa Cruz Biotechnology) and then detected with goat anti-rabbit IRDye 680 (red) and goat anti-mouse IRDye 800CW (green) secondary antibodies, respectively. The blot was imaged with the Odyssey Fc Imager for 2 minutes in each channel. Overlapping ERK (red) and phospho-ERK (green) signals are displayed in yellow. This phospho-ERK1 antibody cross-reacts with phospho-EGFR (upper green band).

Western Blot Cost Comparison

Infrared Detection vs. Chemiluminescence

Cost Savings

Reagents	IR Detection (2 Targets)	Chemiluminescence (1 Target)	Chemiluminescence (strip and reprobe for second target) 2-target total
Secondary Antibody (15 mL) Recommended Dilutions: (1:15,000 for IR#; 1:2,500 for Chemi)	$0.68	$0.33	$0.66
Chemiluminescent Substrate (2 mL)	---	$5.70 (2 mL)	$11.40 (2 mL)
Film (2-4 pieces of film/blot)	---	$7.68	$15.36
Protein Markers Two-color Protein Marker for IR (2 µL) Standard Protein Marker for Chemi (10 µL)	$1.16	$4.68	No charge to reuse marker
Cost Extra Cost Per Blot Compared to IR*	$1.84	$18.39 (2 mL) $16.55	$27.42 (2 mL) $25.58

#IRDye 800CW and IRDye 680RD were used for IR calculations.
*Based on GE Healthcare pricing. September 20, 2011. Assumes 10 x 10 cm blot.

Table 1. Moving to digital imaging with Odyssey infrared imaging technology will save money for your lab. Not only will you save money on reagents, all Odyssey imaging systems eliminate the costs related to film and darkroom expenses.

ODYSSEY® CLx
Infrared Imaging System

The Odyssey CLx is the next generation of the Odyssey Classic, the most trusted and established standard in quantitative Western blot technology.

- The most flexible and multifunctional platform of the Odyssey imaging systems
- Accommodates a wide variety of applications
- Largest imaging surface of all Odyssey imaging systems (25 cm x 25 cm), accommodating up to six microtiter plates

Two Independent Infrared Detection Channels

Two separate lasers and detectors simultaneously detect both fluorescent signals. The optical system employs diode lasers and solid-state detectors with long lifetimes and very low maintenance requirements. Infrared laser excitation outper-

- ☑ Established standard in Quantitative Western blot technology for 10+ years
- ☑ Most versatile Odyssey system for numerous applications
- ☑ Largest imaging surface of all Odyssey systems (25 cm x 25 cm)

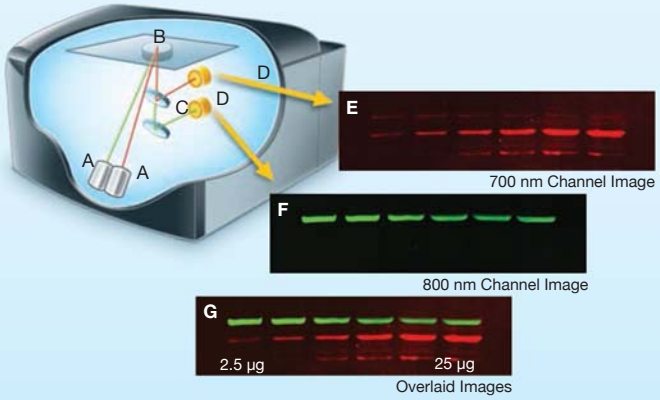


Figure 6. Beams from solid-state 700 nm and 800 nm lasers (A) are focused to form an excitation point on the scanning surface. A microscope objective (B), focused on the excitation point, collects light from both fluorescing infrared dyes. Light from the microscope objective is passed through a dichroic mirror (C) that splits the light into two fluorescent signals. The fluorescent signals travel through two independent optical paths and are focused on separate silicon avalanche photodiodes (D) and detected. In this example, 700 nm fluorescence (IkappaB) is shown in red (E) and 800 nm fluorescence (Tubulin) is shown in green (F). The two colors were imaged simultaneously in a single scan and can be displayed separately or together in a single image (G).*

** Data courtesy of Dr. Catrin Albrecht, IUF, Germany*

forms systems that use white light, LED light sources, and filter wheels by delivering higher intensity excitation light to the fluorophore. A variety of fluorescent dyes and stains are compatible with the 700 nm and 800 nm excitation wavelengths of the two diode lasers in the Odyssey CLx. Spectral overlap is minimized by a 100 nm separation of the two detection channels, and optical filtering ensures that each detector measures fluorescence from only one of the infrared dyes (Fig. 6).

Now Featuring:

AutoScan Function

- Wide dynamic range captures the entire range of data without saturation in a single, time-saving scan – no need for multiple scans to optimize intensity settings
- An even wider dynamic range is available when detecting high-abundance proteins in a single image

Multiple Blot and Plate Scanning

- Simultaneously scan multiple samples of varied intensities in one scan for increased convenience

Easy-to-Use Image Studio Imaging Software

- One-button image acquisition
- Quick user adoption
- Saves time needed to acquire and analyze data

CLx Applications

Western Blots:
Two-Color Infrared and In-Gel

Cell-Based Assays:
In-Cell Western™ and On-Cell Western

Protein Detection:
Coomassie-Stained Gels, Membrane and Slide Arrays

Small Animal Imaging:
In Vivo, Whole Organ, and Tissue Section

Nucleic Acid Detection:
Mobility Shift Assays, DNA Gel Staining (Syto® 60), and Arrays

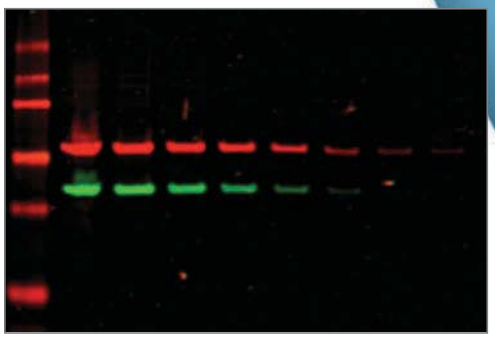
Microwell Assays:
ELISA, Protein Arrays, and RNAi Analysis

MousePOD® Imaging Accessory*

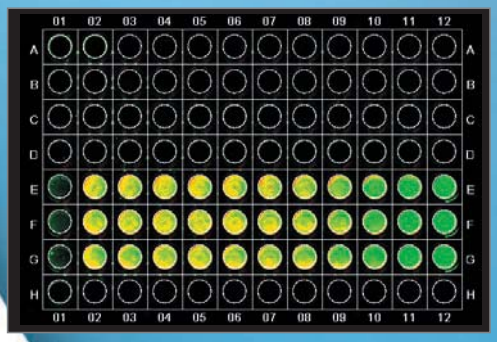
- Fits on the Odyssey CLx scanning surface and accommodates up to three mice or one rat
- Delivers gas anesthesia to animals via nosecones
- Regulates air temperature to maintain animal's temperature during scanning
- Includes small animal imaging module for Image Studio software to quickly mark tumors, organs, and other regions of interest. Pseudo-color display style helps to quickly isolate regions of interest

**MousePOD and anesthesia system are sold separately*

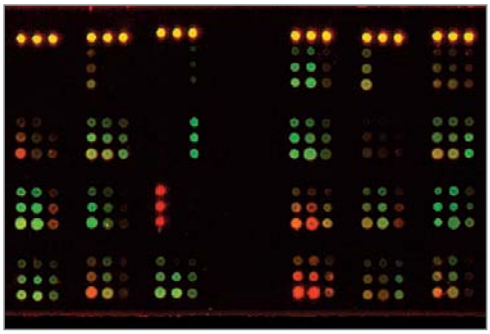
ODYSSEY® CLx Applications



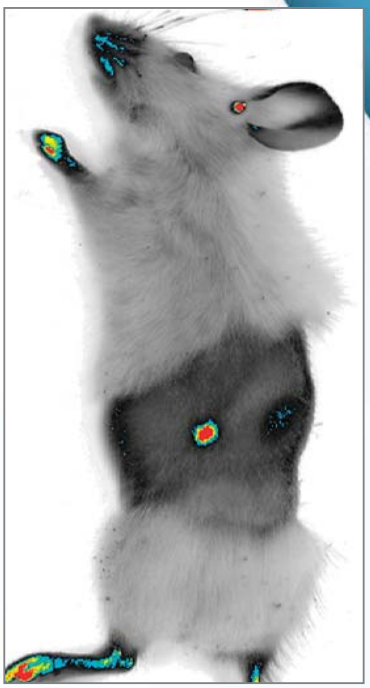
IR Fluorescent Western Blots



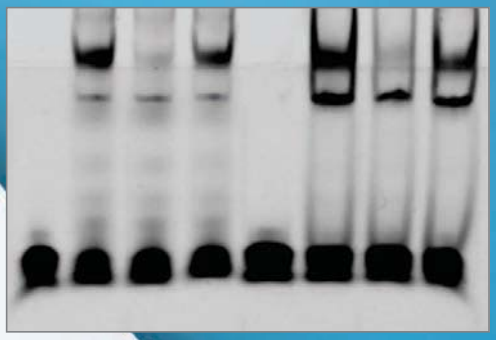
In-Cell Western™ Assays



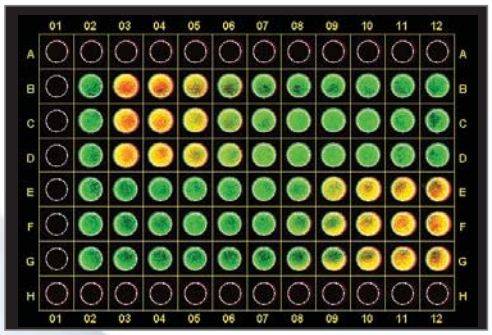
Protein Arrays



Small Animal Imaging

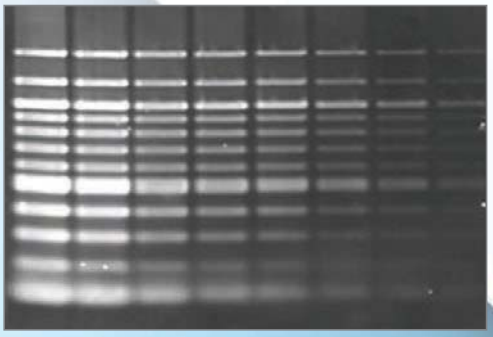


EMSA/Gel Shift Assays

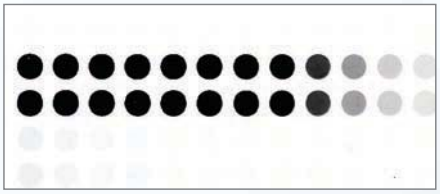


On-Cell Western Assays

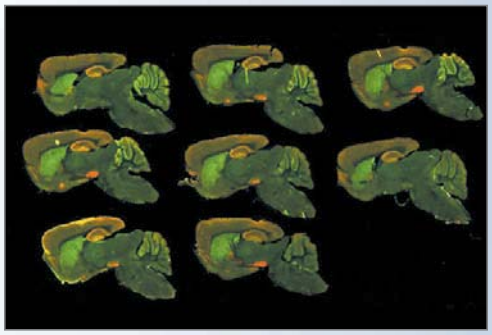
Coomassie-Stained Gels



DNA Gel Staining



ELISA



Tissue Section Imaging

Data courtesy C. Kearns, University of Washington

Odyssey CLx

Odyssey Fc

Odyssey Sa

ODYSSEY® CLx System Specifications

Image Field Size:
25 cm x 25 cm

Dynamic Range:
Manual: 4 logs
Auto: > 6 logs

Laser Lifetime:
40,000 working hours

700 Channel Laser Source:
Solid-state laser diode at 685 nm

800 Channel Laser Source:
Solid-state laser diode at 785 nm

Detectors:
Silicon avalanche photodiodes

Scanning Speed:
5-40 cm/s

Resolution:
21-337 µm

Focusing Range:
Microscope is adjustable 0 mm – 4 mm above the scan bed to obtain best signal-to-noise ratio

Operating Conditions:
15-35°C and dew point no greater than 20°C

Power Requirements:
Universal input range is between 100-240 VAC; 4 Amp maximum; 1 Amp typical; 50/60 Hz

Dimensions:
37 h x 53 w x 62 d cm (14.5 x 21 x 24.4 inches)

Weight:
33 kg (72 lbs)

ETL Listed for US/CAN, CE Marked

Experience Excellence

We know that your time is incredibly valuable, and therefore, the research tools you work with must be reliable, easy to use, and deliver superior results. That's why LI-COR has worked for the past 40 years to innovate in ways that exceed researchers' expectations.

Locations Worldwide

View a complete list of our international distributors at: *www.licor.com/bio/distributors*

U.S.

LI-COR® Biosciences
4647 Superior Street
Lincoln, NE 68504
Phone: 402-467-0700
Phone: 800-645-4267
Fax: 402-467-0819
Email: biosales@licor.com

LI-COR Ltd., UK

Serving UK, Ireland, and Scandinavia

LI-COR Biosciences UK Ltd
St. John's Innovation Centre
Cowley Road
Cambridge
CB4 0WS
United Kingdom
Phone: +44 (0) 1223 422104
Fax: +44 (0) 1223 422105
Email: bio-eu@licor.com

LI-COR GmbH, Germany

Serving Europe, Africa, and the Middle East

LI-COR Biosciences GmbH
Siemensstraße 25A
D-61352 Bad Homburg
Germany
Phone: +49 (0) 6172 17 17 771
Fax: +49 (0) 6172 17 17 799
Email: bio-eu@licor.com

LI-COR®
Experience Excellence

The LI-COR board of directors would like to take this opportunity to return thanks to God for His merciful providence in allowing LI-COR to develop and commercialize products, through the collective effort of dedicated employees, that enable the examination of the wonders of His works.

"Trust in the LORD with all your heart and do not lean on your own understanding. In all your ways acknowledge Him, and He will make your paths straight."

—Proverbs 3:5,6

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