The Path to Quantitative Analysis
Quantitative Westerns Solution Provider

Standard in the Industry
Over 20,000 Users
More than 2,900 Publications
Application Flexibility
Optimized Reagent Solutions
Excellent Technical Support
Easy Transition Protocols
LI-COR Detection System

- 680 & 780 nm solid state laser diodes (A)
  - No spectral overlap
  - Low power consumption
  - Long life lasers (>40,000 hours)
- Silicon avalanche photodiode detectors (C)
- Resolution 21-337 microns
- Internal hard drive
- Secure network connectivity
Why Use Infrared Fluorescence?

- Many natural and synthetic compounds autofluoresce in the visible region
  - Nitrocellulose, PVDF, nylon, cellular content
- Low background fluorescence in IR range translates to excellent sensitivity
Applications
Odyssey® Application Flexibility

Western Detection
Two-Color Blots • In-Gel Westerns

Cell-Based Assays
In-Cell Western™ Assay • On-Cell Western™ Assay • RNAi

Microwell Assays
FLISA • Transcription Factor Arrays

Protein Gel Documentation
1D/2D • Coomassie

Nucleic Acids
EMSA/Gel Shift • DNA Staining

Small Animal Imaging
Organ + Tissue Section Imaging
Easy Transition From Chemiluminescent

Perform electrophoresis and transfer as usual
↓
Block in Blocking buffer 30 minutes to an hour
↓
Dilute PRIMARY Antibody and incubate as usual
↓
Wash
↓
Dilute IRDye® labeled SECONDARY Antibody and incubate 1 hour
↓
Wash
↓
Blot is ready for imaging

* Easy transition from ECL *
Direct Infrared Detection

Antigen on membrane

Primary antibody binds to antigen

IRDye labeled secondary antibody binds to primary antibody

ON-SCREEN Display

INFRARED APD DETECTOR

INFRARED LASER DIODE
Direct Infrared Detection
Quantitative Western

Tf protein:

Dilutions of transferrin blotted on nitrocellulose, detected with rabbit anti-Tf primary and IRDye® secondary antibody

1. No exposure/integration time - No darkroom
2. No enzyme kinetics - Not time dependent; no substrates
3. Large linear range of chemistry
4. No signal diffusion - Band clarity
5. Sensitivity equivalent or better than chemiluminescence
**Quantification Accuracy - Linearity**

Linear range is the range over which bands can be accurately quantified, in contrast to dynamic range which is the total range of the detection hardware.

Two-fold serial dilutions of antibody (6 ng to 0.19 ng) were spotted on nitrocellulose (above). $R^2$ from 3.0 ng to 0.37 pg was 0.998, demonstrating excellent linearity even at low concentration.

$R^2 = 0.998$

>4 logs linear range
Linearity Data

Odyssey® – Direct Infrared Detection

Signal IS proportional to amount of antigen

0.3 pg-0.6 ng detection limit (all 15 protein spots)

$R^2 = 0.998$
Two-Color Simultaneous Detection

This shows phospho-EGF (Red, 700 Channel) normalized to Actin (Green, 800 Channel) in A-431 cells treated with a dose response of EGF.

* Two-color detection requires primary antibodies from different hosts
Monitoring EGF Receptor Phosphorylation

Anti-EGFR and anti-phospho-EGFR antibody specificity in A431 cells

The mobility shift caused by phosphorylation is visible (A) as indicated by the red bands above the yellow bands. (yellow indicates overlapping red and green signals).

Single color images (B and C) can be overlaid (A) to show both total protein and phosphorylated protein.
The Odyssey *In-Cell Western (ICW) Assay* is a high-throughput approach to simultaneously detect and quantify two separate proteins directly within cells.
Normalized level of EGFR phosphorylation was used to generate IC-50 curve for PD168393.

Data one target (EGFR) and PD168393; 800 channel = normalization; 700 channel = experimental.
Optical Agent Binding Assay:
- A dose response was noted when A431 cells were incubated with increasing concentrations of IRDye 800CW EGF (green). Cells were normalized to TO-PRO-3 staining (red).

Optical Agent Competitive Challenge:
- Increasing concentrations of unlabeled EGF successfully competed for binding sites with IRDye 800CW EGF (green). Cells were normalized to TO-PRO-3 staining (red).
Molecular Imaging - Tissue Sections

Femurs

Vertebrae cross-section

21 um Odyssey scans of bone sections
IRDye 800CW BoneTag deposition presented in green and autofluorescence in red.
Additional Applications

- **Protein Detection**
  - In Gel Western
  - 1-D / 2-D Coomassie®

- **Nucleic Acid Detection**
  - EMSA / Gel Shift Assays
  - DNA Staining

- **Microwell Assays**
  - ELISA/FLISA
  - Transcription Factor Arrays

- **Protein Arrays**
  - Plated based
  - Membrane based
  - Slide based
  - Quansys Multiplex ELISA (cytokine/chemokines)
  - Biosciences GE Array™
EMSA / Gel Shift Assays

- IRDye® labeled DNA fragments
- Data based on the interaction of a protein and DNA fragment
- Shift in mobility is visualized when scanned
Characterization of Distinct Stat5b Binding Sites That Mediate Growth Hormone-stimulated \textit{IGF-I} Gene Transcription

\textbf{A}  

\begin{tabular}{|c|c|c|c|c|c|c|c|c|}  
\hline  
5' distal RE-1 (nM) & 0.5 & 1.0 & 1.5 & 2.0 & 2.5 & 3.0 & 3.5 & 4.0 & 4.5 & 5.0 & 5.5 & 6.0 & 6.5 & 7.0 & 7.5 \\  
\hline  
\end{tabular}

\textbf{B}  

\begin{tabular}{|c|c|c|c|c|c|c|}  
\hline  
HS7 GHRE-1 (nM) & 1.25 & 2.5 & 5.0 & 7.5 & 10 & 12.5 & 15 & 20 & 25 \\  
\hline  
\end{tabular}

\begin{align*}  
\text{Kd} &= 0.02 \text{ nM} \\
\text{r}^2 &= 0.97 \\
\text{Kd} &= 0.82 \text{ nM} \\
\text{r}^2 &= 0.99 \\
\end{align*}
Odyssey® Application Flexibility

Advancing Discovery with Infrared Imaging

Applications for the Odyssey® Infrared Imaging System

A wide-range of applications benefit from near-infrared fluorescent detection on the Odyssey System

Our applications profiles are designed to provide you with examples, support information, recent publications, and available on-line subject-specific webinars.

- Coomassie Gels
- DNA Gel Staining
- ELISA/FUISA
- EMSA/Gel Shift Assay
- Glycoprotein Detection
- In-Cell Western™ Assay
- In-Gel Western Assay
- Northern Blot
- On-Cell Western Assay
- Protease Assay
- Protein Array
- Quantitative Western Blot
- Reporter Gene Assay
- Reverse Phase (Lysate) Array
- RNAi Screens
- Small Animal Imaging
- Southern Blot
- Tissue Section Imaging
- Transcription Factor Assay
- Microweset

http://www.licor.com/bio/
Solutions for Applications
Optimized Reagent Solutions

- Optimized Kits for Western Blots
- Optimized Kits for Cell-Based Assays
- EMSA Buffer Kit
- Optical Probes for Tumor Imaging
- Labeling Kits for Antibodies, Proteins
- Multiple Infrared Dyes
- Labeled Antibodies and Conjugates
• Detection of other dyes is possible

• Many different conjugates are available to meet research needs

• Use may be limited to one channel depending on wavelength of chosen dye
IRDye® Conjugated Antibodies and Reagents

- IRDye700DX Conjugates
- IRDye800 Conjugates
- IRDye800CW Conjugates
Software Solutions

- Instrument software
  - Administration
  - Diagnostics
- Application software
  - Imager control
  - Background subtraction
  - Band finding and sizing
  - Quantification
  - ICW % response calculations (including normalization)
  - Small animal imaging analysis
Application Protocols
Application Protocols
Get the help you need, when you need it.

http://www.licor.com/bio/support
Western Blotting - Direct Fluorescent Detection

Antigen on Membrane

Primary antibody binds to antigen

IR-labeled secondary antibody binds to primary antibody

Infrared dye (IRDye680 LT, IRDye800CW)

SCAN ON ODYSSEY -

Time not critical (days, weeks, months).
No substrates required.
No film / darkroom required.
Odyssey® Western Protocol

Protein Gel Electrophoresis

Electro-transfer on membrane (low background PVDF, NC)

Blocking
(Try Odyssey Blocking buffer)

Hyb. With Primary Antibody
1:1000 to 1:5000

Hyb. With Infrared (IR)-labeled Secondary Antibody
1:15,000 (1:5000 ~ 1:25,000)

Scanning with Odyssey
Factors That Alter the Performance of a Western Blot

A low background membrane is essential for fluorescent WB success.

A. PVDF Membrane:

- **Millipore Immobilon™ FL**
  - 700 nm Channel
  - 800 nm Channel

- **Millipore Immobilon™ P**
  - 700 nm Channel
  - 800 nm Channel

- **BioRad Immun-Blot®**
  - 700 nm Channel
  - 800 nm Channel

- **Amersham Hybond™-P**
  - 700 nm Channel
  - 800 nm Channel
Factors That Alter the Performance of a Western Blot

Antibody performance can sometimes be compromised by the blocker chosen.

B. Blocking Buffer:

Western blots detected with anti-PKCα and IRDye 800CW Goat anti-mouse.

T293 Cells Stimulated with TGF- at 0, 2.5, and 5 min
Factors That Alter the Performance of a Western Blot

C. Miscellaneous Contamination:

A) Dirty transfer pad

B) Acrylamide on membrane

C) Coomassie contaminated container

D) Blue ink pen

E) Fingerprint

F) Bacterial contamination in primary antibody
Scanning Procedures
File/New... 開啟新檔案...

<No Project>

New Project
Path: D:\Mandy Profile\My Documents\Licom\Odyssey\Projects\Training Test

Done Import Scan... Scan... Cancel

Scanner Login
Scanner: LI-COR Odyssey (ODY-1723)
User Name: unimed
Password: 
OK Cancel

Tutorial Project
QuantScan Scans
FirstScan
SizingScan
Original Analysis
Sizing Analyses
MyAnalysis
Membrane / gel 正面朝下, 上方靠近操作者, 長的一邊橫放
Plate 正面朝上，緊靠 Alignment Guide 放置
LI-COR Presets:

<table>
<thead>
<tr>
<th>LI-COR Presets</th>
<th>Membrane</th>
<th>DNA Gel</th>
<th>Protein Gel</th>
<th>Microplate2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
</tr>
<tr>
<td>Quality</td>
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</tr>
<tr>
<td>Focus Offset</td>
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<td>2.0</td>
<td>0.5</td>
<td>3.0 mm</td>
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<tr>
<td>Channels</td>
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<td>700, 800</td>
<td>700, 800</td>
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<tr>
<td>Intensity</td>
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<tr>
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</tr>
<tr>
<td>Scan Size</td>
<td>10,10</td>
<td>10,10</td>
<td>10,10</td>
<td>13,9</td>
</tr>
</tbody>
</table>
Click and hold down the mouse button in the lower left corner of the area to be scanned.

Drag the cursor to the upper right corner of the area to be scanned and release the mouse button.
顯示剩餘之時間

顯示已完成之百分比

影像即時顯示
**Save As Scan-Analysis**

- **Scan Name:** 2007-06-29-165738
- **Analysis Name:**

**New Analysis**

- **Name:** Original Analysis
- **Description:**

Any image manipulation operations performed within the New Analysis dialog are applied to the acquired image when the OK button is pressed. The original image data from the scan is replaced by the altered data.

**Analysis Image**

- **Adjust Image Curve...**
- **Select Image Display...**

**Buttons:**
- OK
- Cancel
Post-Scanning

掃描影像檢視...

Double click
掃描影像檢視...

主工具列

影像對比度調整
影像顯示大小調整
700/800 單色畫面轉換
雙色/單色畫面轉換
彩色/灰階畫面轉換

開啟新掃描
Post-Scanning
Post-Scanning

掃描影像輸出... File/Export Image->
Thank You!

Western Detection
Two-Color Blots • In-Gel Westerns

Cell-Based Assays
In-Cell Western™ Assay • On-Cell Western™ Assay • RNAi

Microwell Assays
FLISA • Transcription Factor Arrays

Protein Gel Documentation
1D/2D • Coomassie

Nucleic Acids
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Organ + Tissue Section Imaging