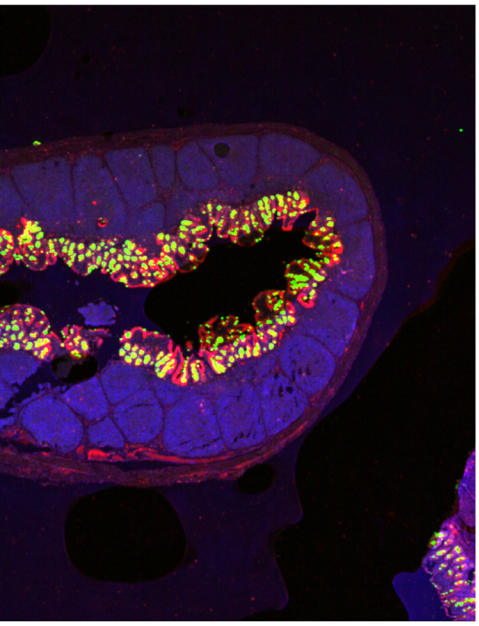
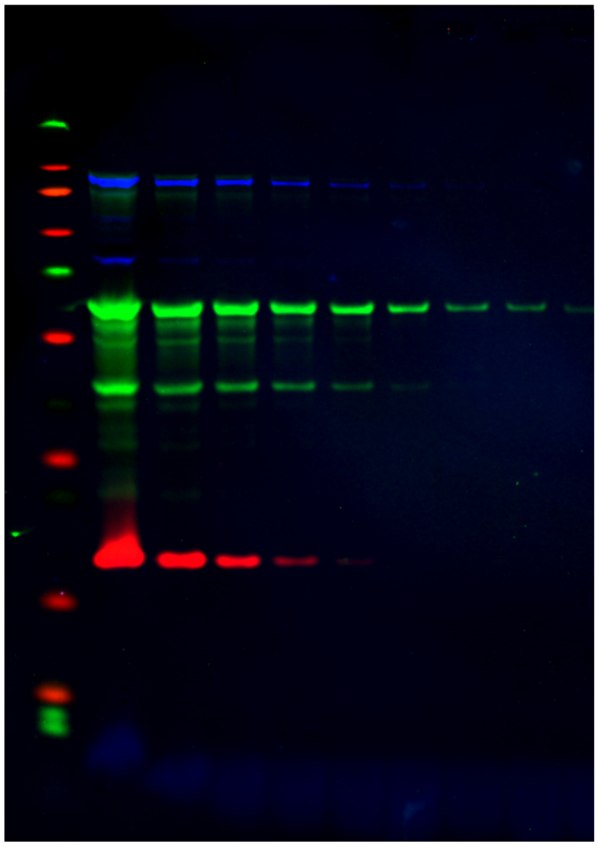
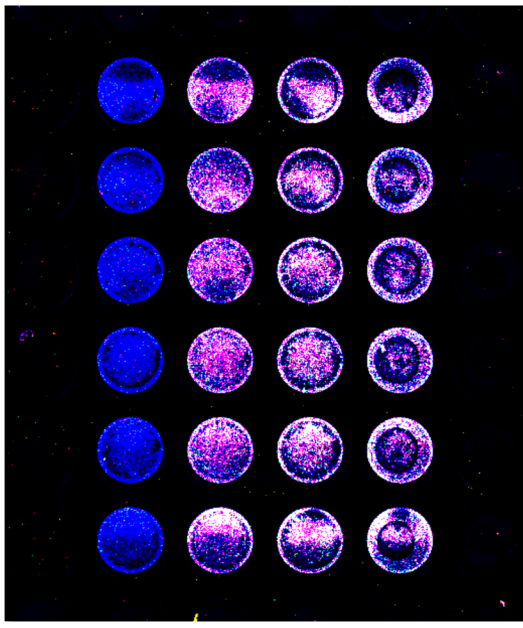
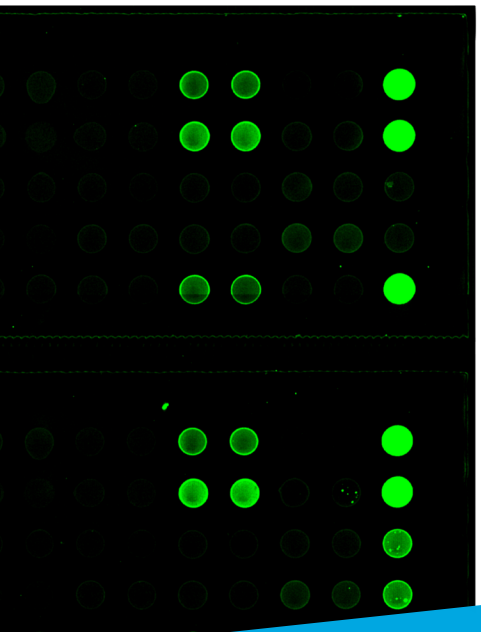
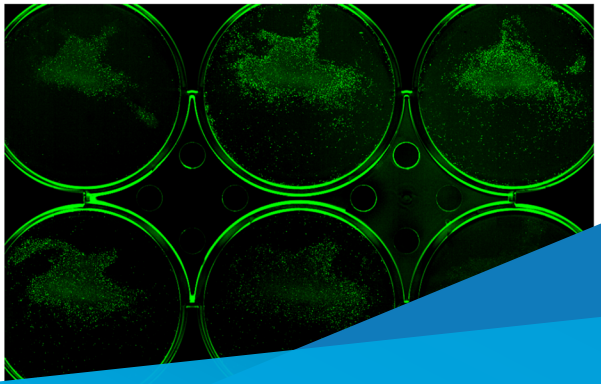
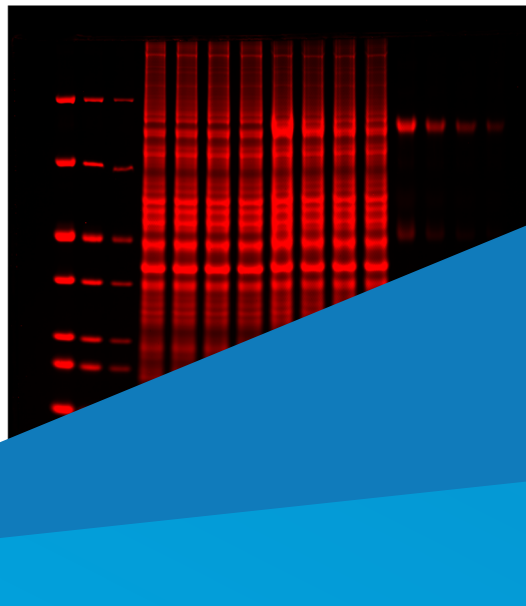
Applications for Advancing Research

Odyssey® Family Imaging Systems

LI-COR®

What will your research teach you?

LI-COR Odyssey® Imaging Systems deliver high-quality data for the applications you need to advance your research and make breakthrough discoveries. The Odyssey XF Imager covers your essential applications, while the Odyssey DLx enables a host of near-infrared fluorescent applications. With the Odyssey M, explore more than a dozen applications and up to 18 fluorescent and luminescent channel combinations to power your scientific advancement forward.

Table of Contents

Membrane Applications

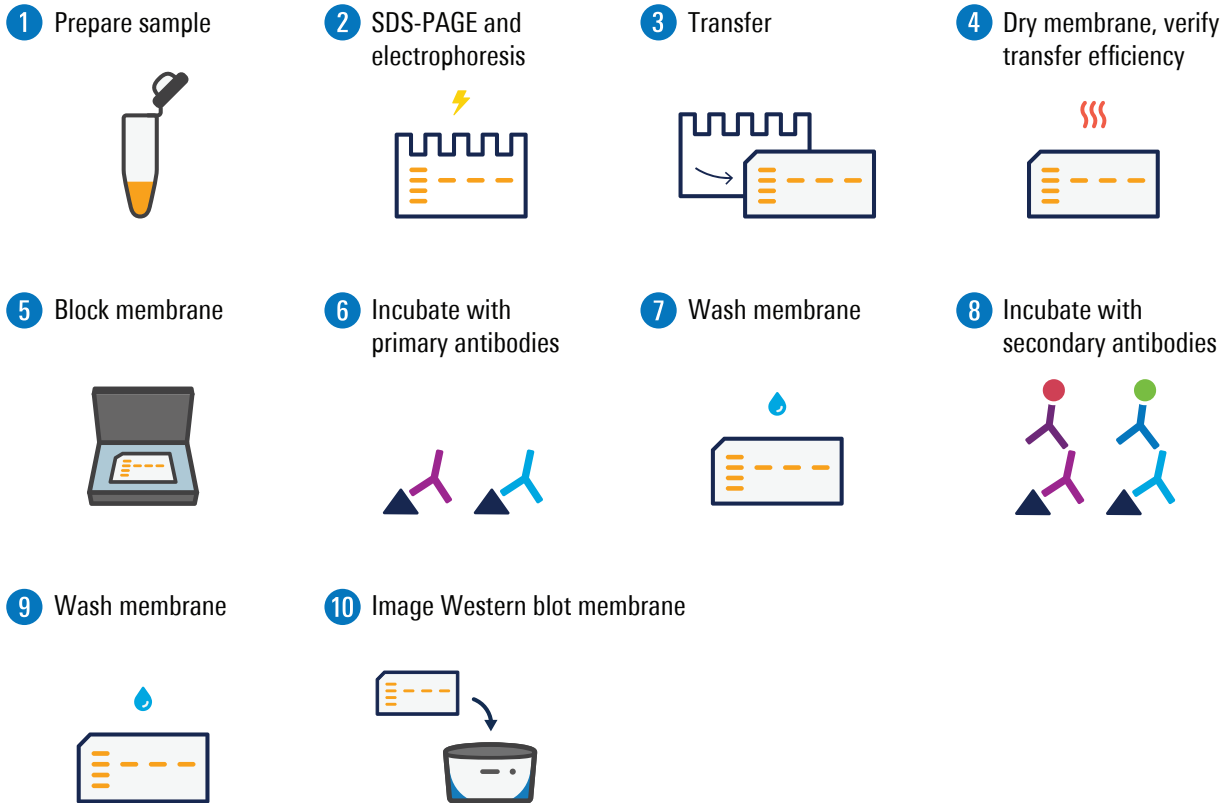
Multi-Well Plate Applications

Slide Applications

Gel Applications

Quantitative Western Blots

Typical Western Blot Workflow



Western blotting is a fundamental and widely used assay to detect specific proteins. Quantitative Western blotting enables accurate measurement of protein expression changes between different conditions and treatments from different sample types. Typical detection methods for a quantitative Western blot are near-infrared fluorescent and visible fluorescent detection.

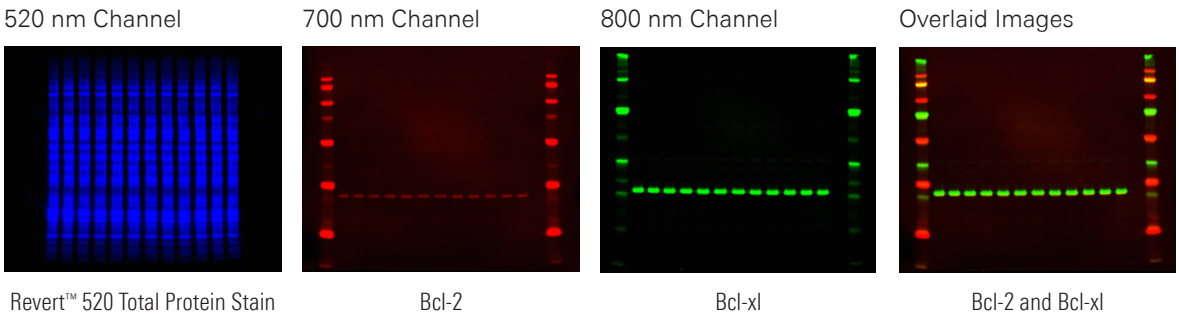


Figure 1. Bcl-2 and Bcl-xl expression was decreased in etoposide-treated Jurkat lysate when normalized to Revert 520 Total Protein Stain. In a sample experiment, 10 µg of Jurkat lysate was loaded per well. Samples 1 – 4 were untreated controls, while samples 5 – 8 and 9 – 12 were treated with 50% and 100% etoposide, respectively. Bcl-2 was detected in the 700 nm channel, and Bcl-xl was detected in the 800 nm channel. Signal was normalized to Revert 520 Total Protein Stain detected in the 520 nm channel. Images were acquired using an Odyssey® M Imager.

Qualitative Western Blots

Where a quantitative Western blot can tell you how much of a specific protein there is, a qualitative Western blot can identify whether a specific protein is present or not. A qualitative Western blot is useful for yes/no answers and can be performed using fluorescent, colorimetric, or chemiluminescent detection.

Qualitative Western blot | Identify the presence or abscence of a specific protein of interest.

Quantitative Western blot | Identify how much of a specific protein of interest is present.

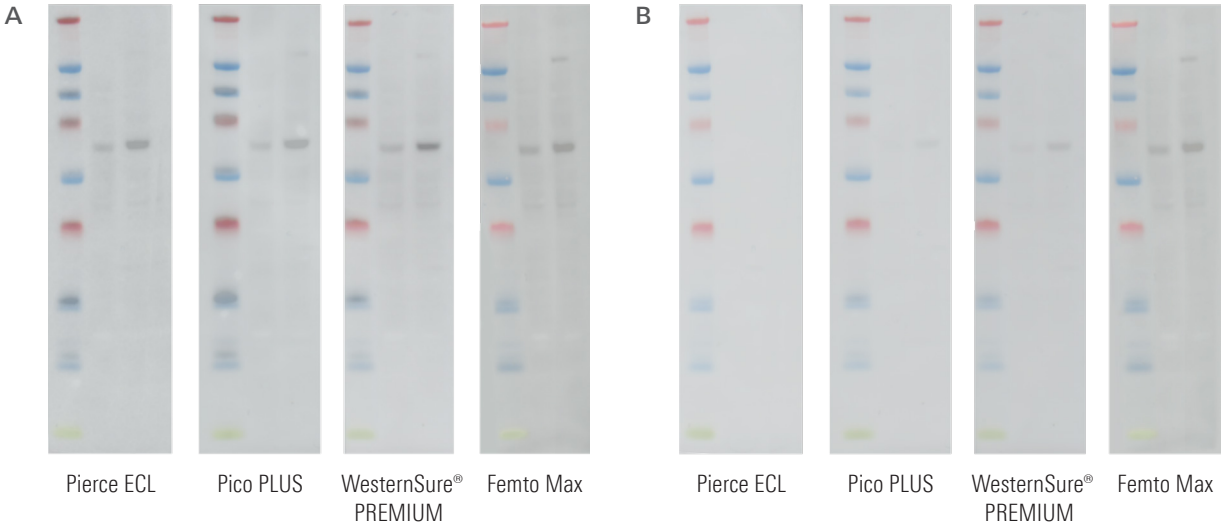
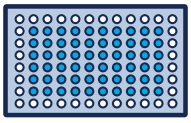


Figure 2. Detection of pAkt in various substrates using chemiluminescent Western blot detection. Western blots were performed using two Jurkat lysates—a negative control in lane 2 and a positive control in lane 3. Molecular weights were marked by WesternSure® Pre-Stained Chemiluminescent Protein Ladder. Detection of pAkt was done using p-Akt mouse monoclonal primary antibody and WesternSure Goat anti-Mouse Secondary Antibody in various substrates with Intercept® (PBS) Blocking Buffer, including Pierce ECL Western, SuperSignal West Pico PLUS, WesternSure PREMIUM Chemiluminescent Substrate, and SuperSignal West Femto Max. Images were acquired using an Odyssey® M Imaging System. Display settings were optimized individually for panel A images and were linked for panel B images.

In-Cell Western™ Assay

Typical In-Cell Western Assay Workflow

1 Culture cells



2 Stimulate or inhibit cells

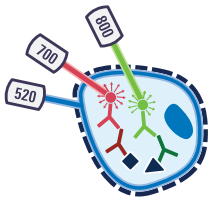
3 Fix and permeablilize cells

4 Incubate with blocking buffer

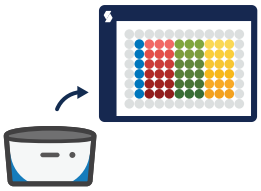
5 Incubate with primary antibody

6 Incubate with secondary antibody and CellTag™ 520 Stain

7 Excite with infrared lasers



8 Acquire image using Odyssey Imager



The In-Cell Western Assay is a higher-throughput, accurate way to examine and quantify proteins in fixed, permeabilized cells using 96- or 384-well microplates. The In-Cell Western Assay provides a snapshot of cells within a more native context. It is a valuable tool to assess infectivity and neutralization in virology studies and to quickly characterize cell signaling parameters for the development of targeted therapeutics.

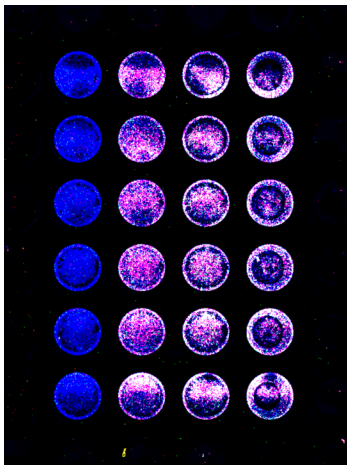


Figure 3. Anisomycin exposure duration influences phosphorylation of SAPK/JNK and p38, steering the pathway toward apoptosis. Approximately 13,000 HeLa cells were loaded into each well and exposed to 1 µg/ml of anisomycin for varying durations. Column 1 was used for background and normalization to CellTag™ 520 Stain. Column 2 was an untreated control. Columns 3 and 4 were treated with anisomycin for 5 and 10 minutes, respectively. pSAPK/JNK was detected in the 800 nm channel, and pP38 was detected in the 700 nm channel using an Odyssey® M Imager.

On-Cell Western Assay

The On-Cell Western Assay is a higher-throughput and accurate way to examine and quantify cell-surface proteins in fixed cells using 96- or 384-well microplates. The On-Cell Western Assay is similar to the In-Cell Western™ Assay, but cells are not permeabilized. This assay can also be used to quantify ligand binding to cell-surface receptors in the development and manufacturing of targeted therapeutics.

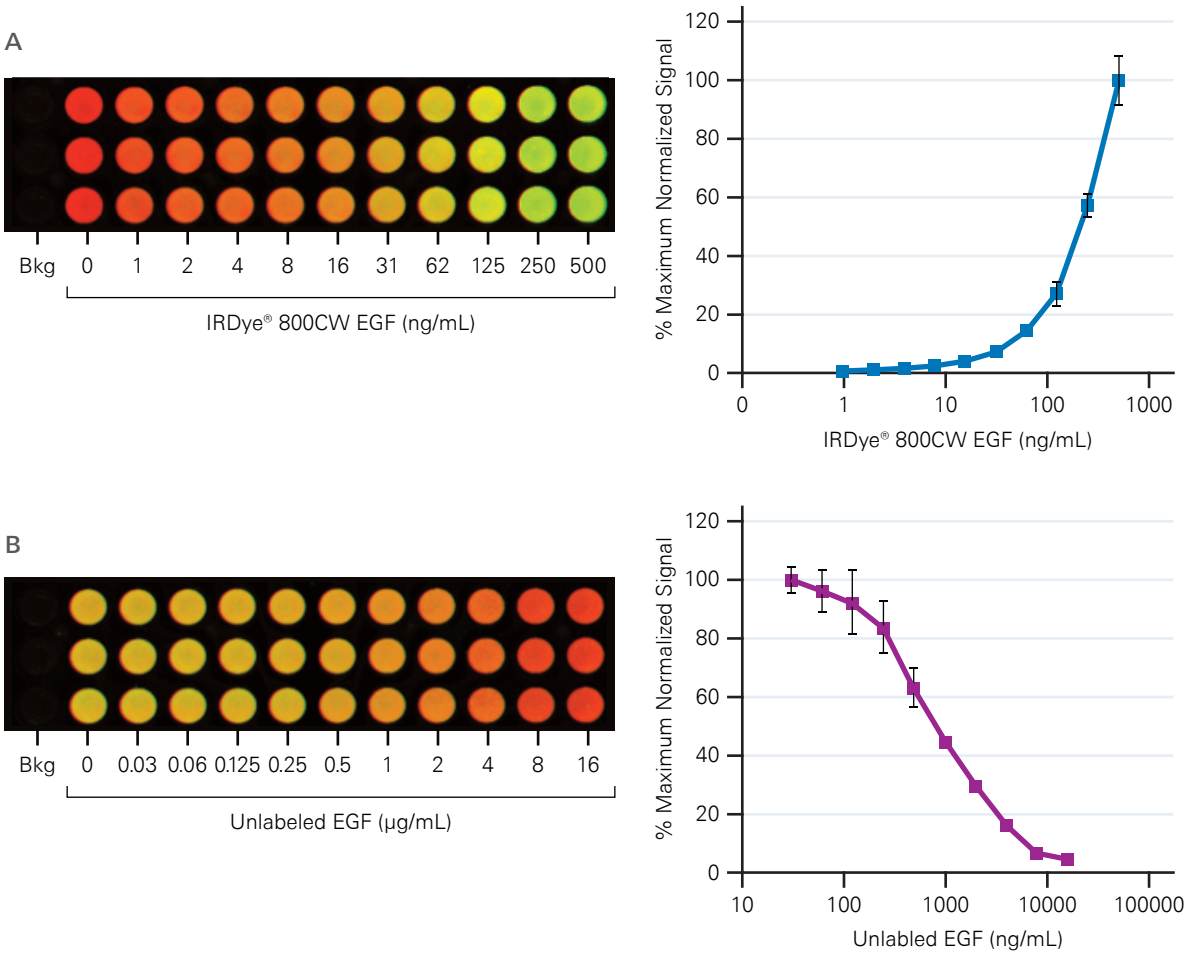
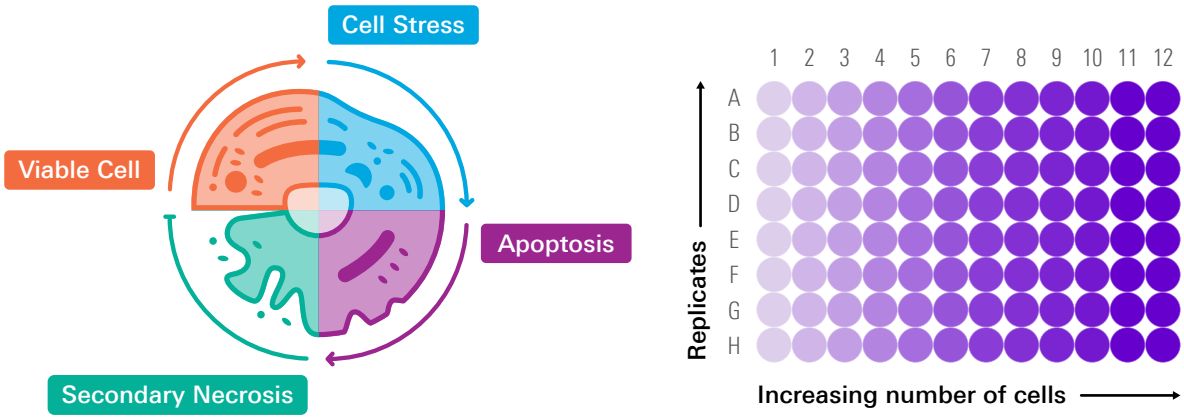


Figure 4. Binding specificity of EGF to EGFR is observed using a competition assay with unlabeled EGF and EGF labeled with IRDye® 800CW (IRDye 800CW-EGF). IRDye 800CW-EGF was detected in the 800 nm channel (green) and normalized to cell number measured using CellTag™ 700 Stain detected in the 700 nm channel (red). A) Wells were treated with increasing concentrations of IRDye 800CW-EGF, and a corresponding increase in normalized 800 nm signal indicated an increase in binding. B) IRDye 800CW-EGF binding is blocked with increasing concentrations of competing unlabeled EGF, demonstrating specific binding of unlabeled EGF.

Cell Health Assay

Cell analysis involves antibody- and reporter-based cell health assays performed on populations of intact cells using a microplate. Various cell analysis detection methods can be used, including fluorescence, luminescence, and colorimetric detection. These wavelengths are compatible with detection of green fluorescent protein, luciferase-based assays, and other protein reporters.



Cell analysis allows researchers to monitor entire cell populations and answer questions about cell health and biological activity. This data provides the information researchers need to understand and predict cellular states and responses to different treatments and conditions.

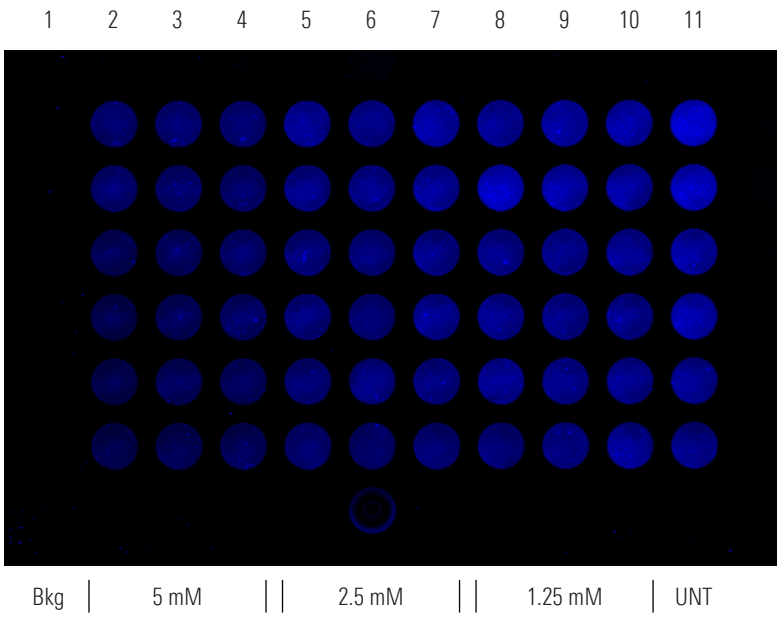
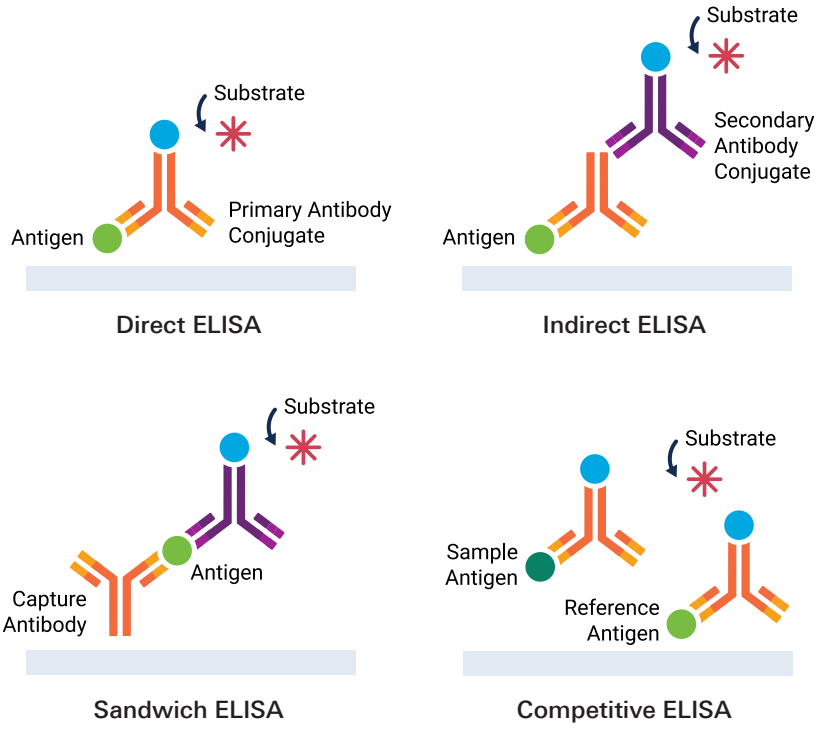


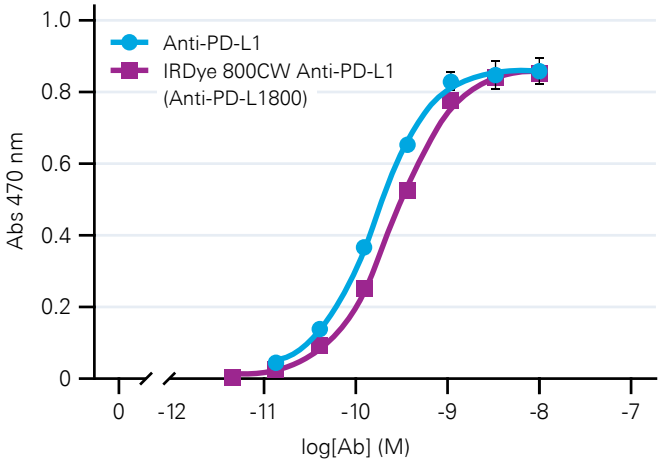
Figure 5. An example cell health assay to monitor NIH3T3 cells treated with anisomycin. NIH3T3 cells were plated and treated with a dilution of anisomycin in serum free media and incubated overnight. Columns 2-4 were treated with 5 mM anisomycin, columns 5-7 with 2.5 mM, and columns 8-10 with 1.25 mM. Column 11 was left untreated. The cells were then stained with crystal violet and imaged using the 535 Trans channel of an Odyssey® M Imager.

ELISA

The enzyme-linked immunosorbent assay (ELISA) is a plate-based method for detecting and quantifying substances and biological samples (e.g., proteins, peptides, and antibodies). ELISAs are a reliable way to detect proteins or analytes in a larger, more complex mix, such as a cell and tissue lysates or serum samples.

This flexible assay has multiple methods and can be performed using colorimetric detection in 96- or 384-well microplates.



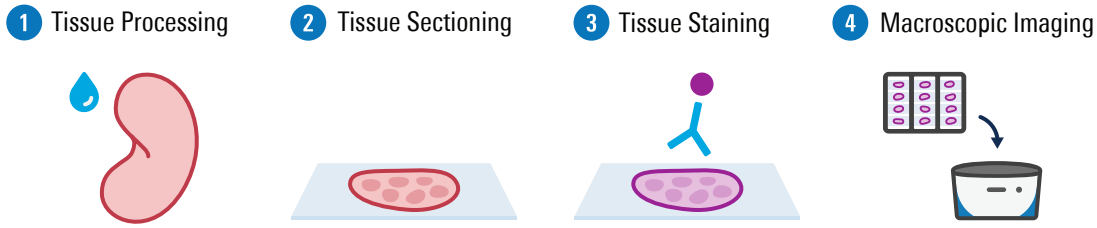


Channel	EC_{50} Anti-PD-L1	EC_{50} (Anti-PD-L1800)
470 Trans	0.161 nM	0.256 nM

Figure 6. OPD detection of an ELISA plate was assessed using an Odyssey® M Imager. Anti-PD-L1 and Anti-PD-L1800 antibodies were evaluated for binding to PD-L1/Fc by indirect ELISA. Anti-PD-L1 or Anti-PD-L1800 antibodies were briefly incubated in wells coated with PD-L1, followed by incubation with an HRP-secondary antibody. OPD substrate was added to each well, incubated for 20 minutes, and quenched using dilute sulfuric acid. Images were acquired using the 470 Trans channel of an Odyssey® M Imager. Data were analyzed in GraphPad Prism with a 4-parameter dose-response equation to extract EC_{50} values.

Tissue Section Imaging

Preparation and Imaging of the Tissue Section



Tissue section imaging is done to examine cross-sections of *ex vivo* tissue to determine the presence or absence of diseased tissue, such as cancer, or to assess the efficacy, specificity, toxicity, localization, and biodistribution of a therapeutic.

Tissue imaging is a vital step for detailed identification of targeting agent locations within an organ, such as the cellular localization of nucleic acids or any protein of interest. Consecutive tissue sections allow for analysis of changes at different tissue depths, and imaging of multiple slides at once lets you screen slides and triage for downstream microscopy analysis.

Histological staining is used to create contrast in tissue features. Histological stains, such as H&E stain or Alcian blue, allow for the study of tissue structure and help identify the presence or absence of diseased tissue at the macroscopic level.

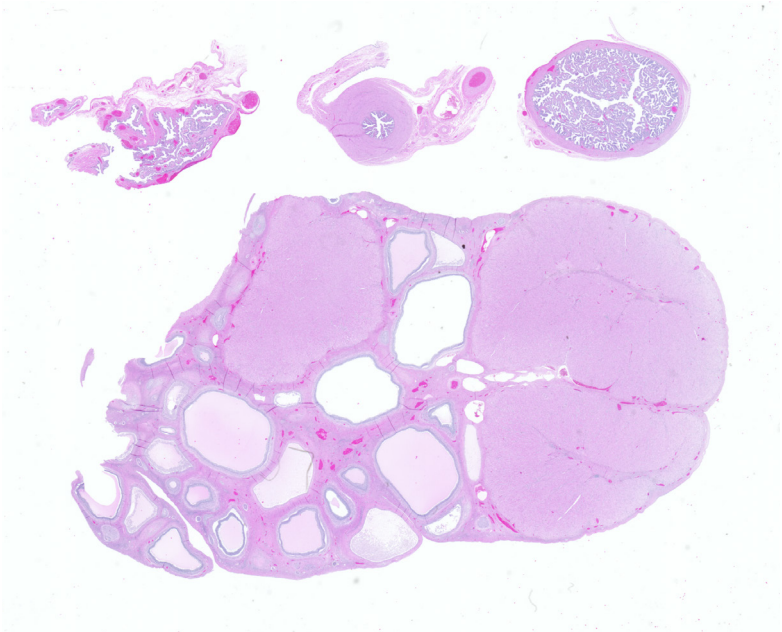


Figure 7. An example tissue section using a pig ovary. Ovaries were deparaffinized, stained using H&E stain, then imaged at 5 µm using the RGB Trans channel of an Odyssey® M Imager.

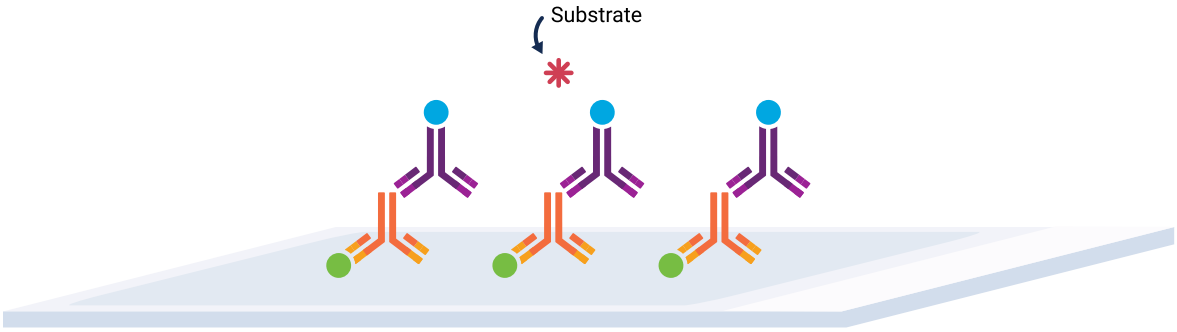
Protein Array

Protein arrays are a high-throughput way to generate information about protein abundance or modification state. Protein arrays allow researchers to analyze a large number of samples simultaneously with many replicates and help conserve precious samples. There are three primary protocols for protein microarrays:

Reverse phase lysate arrays: Spot proteins on slides. Add primary antibodies and secondary antibodies. Then image.

Antibody arrays: Spot capture antibodies in a grid. Dilute cell lysates, mix sample with detection antibodies, and incubate them with a commercially available array. Then image.

Microwestern arrays: Spot samples on acrylamide slabs in a 96-well array. Electrophorese, transfer to a membrane, and place in 96-well gasket device. Then image.



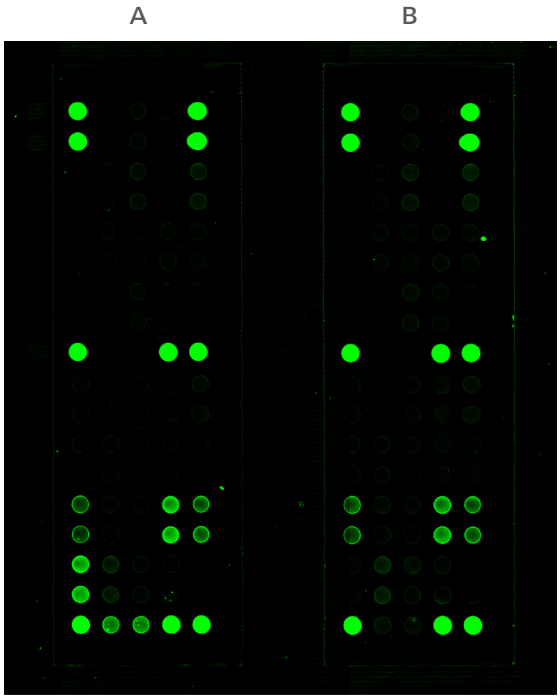
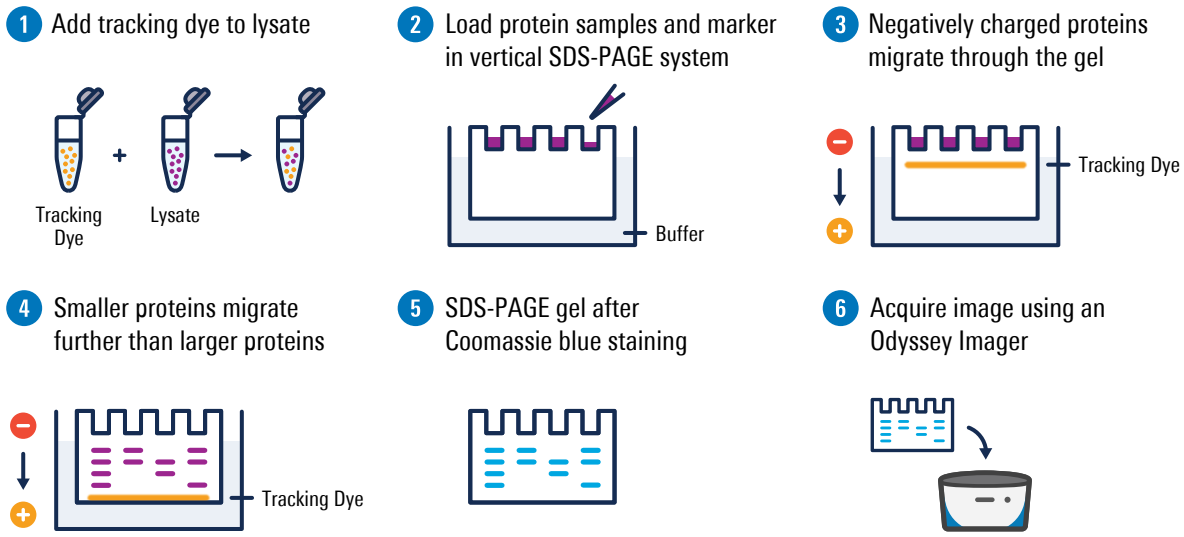


Figure 8. An example antibody array used to assess protein abundance in cell lysates. The left pad (A) was incubated with lysate using SH-SY5Y cells treated with retinoic acid. The right pad (B) was incubated with lysate using SH-SY5Y cells treated with TPA (12-O-tetradecanoyl-phorbol-13 acetate). The lysate was removed, and the slide was incubated with biotinylated antibodies from the PathScan® kit. The slides were then incubated with IRDye® 800CW Streptavidin (1:5000) and detected on an Odyssey® Imager.

Protein Gel

Preparation of the Protein Gel



A protein gel can be used to analyze electrophoretically separated proteins while the proteins are in the gel. Protein gels are often used to assess protein purity and to detect degradation products in forced degradation studies for the development of therapeutics.

A protein gel can also be run separately to supplement the findings of an immunoblot. A wide variety of fluorescent and colorimetric stains can be used for detection.

Fluorescent Stains

- Coomassie Blue
- SYPRO® Ruby
- SYPRO® Orange
- Phosphoprotein stains
- Glycoprotein stains

Colorimetric Stains

- Coomassie Blue
- Silver stains

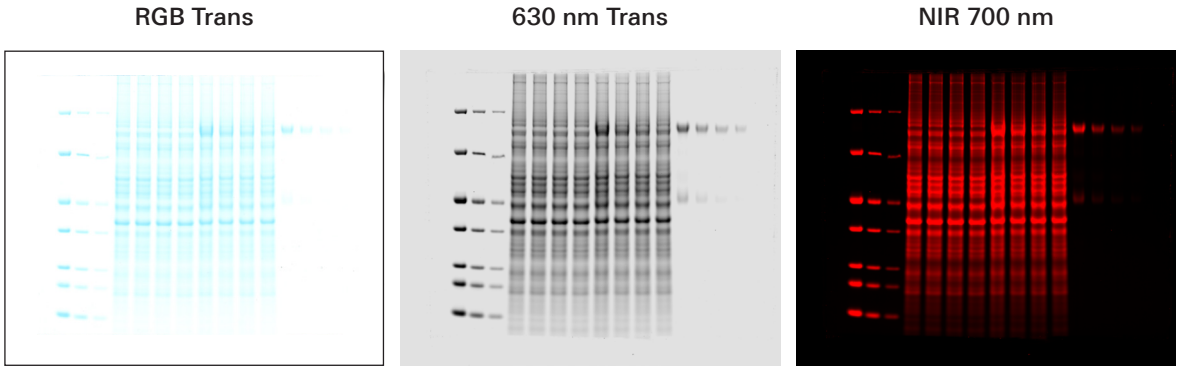
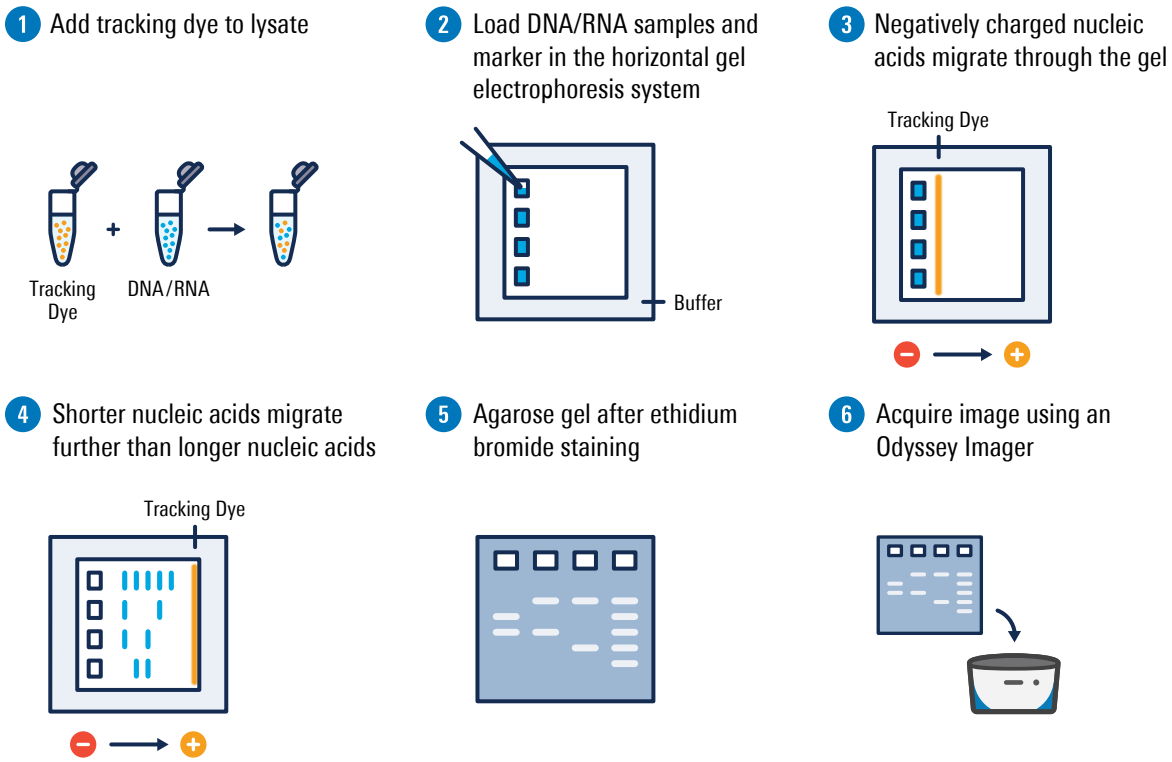


Figure 9. An example protein gel stained with Coomassie SimplyBlue™ SafeStain imaged in multiple channels. The gel was imaged using the RGB Trans, 630 nm Trans, and NIR 700 nm channels of an Odyssey® M Imager. Lanes 1 – 3 contain an unstained molecular weight marker. Lanes 4 – 7 were loaded with 10 µg C32 whole cell lysate. Lanes 8 – 11 were loaded with hemagglutinin protein spiked into 10 µg C32 lysate. Lanes 12 – 15 contained only the hemagglutinin protein.

Nucleic Acid Gel

Preparation of the Nucleic Acid Gel



A nucleic acid gel is an assay to analyze, with sub-nanogram sensitivity, electrophoretically separated DNA or RNA in a gel. A nucleic acid gel allows for labeling, visualization, and possible excision of separated nucleic acids according to size. Fluorescent dyes, such as SYBR™ Safe, SYTO® 60, and GelRed®, are safer and more sensitive than ethidium bromide, but ethidium bromide is still often used for detection.

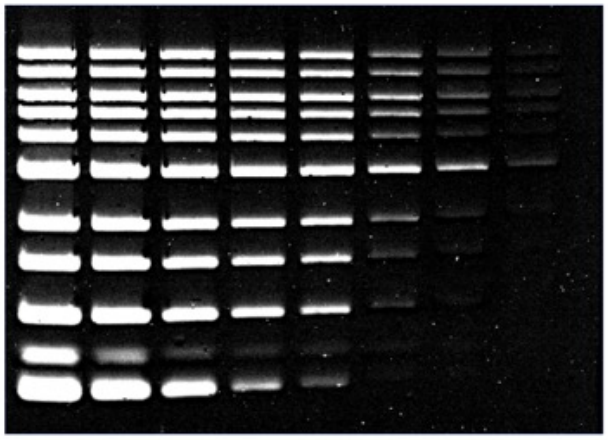
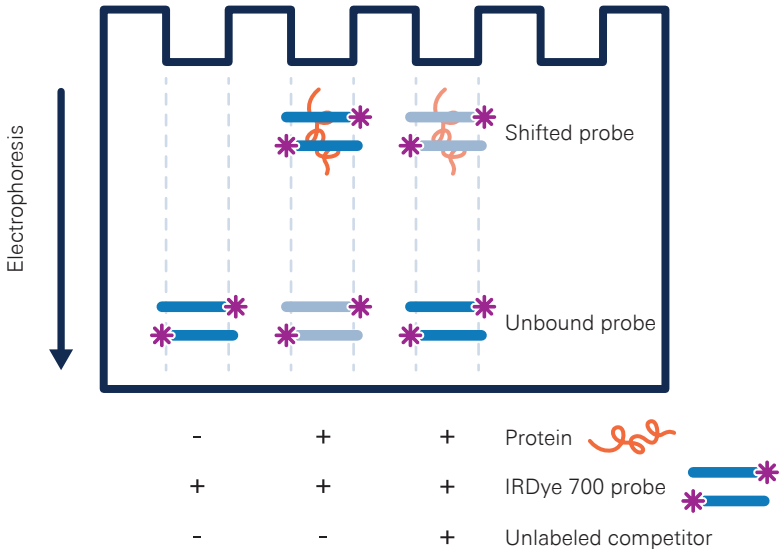


Figure 10. An example nucleic acid gel. A two-fold serial dilution of 1kb ladder (NEB) was run in a standard 1% agarose gel then stained using GelRed® Nucleic Acid Gel Stain. Images were acquired in the 520 nm channel using an Odyssey® M Imager.

EMSA/Gel Shift Assay

EMSA/Gel Shift Migration



An EMSA/gel shift assay is used to study protein:nucleic acid complexes and interactions and can be used to analyze the systems responsible for many cell-level processes, including transcription, replication, and repair. Fluorescent detection of an EMSA offers a fast and sensitive method to get results without the hazards of performing a radioactive EMSA.

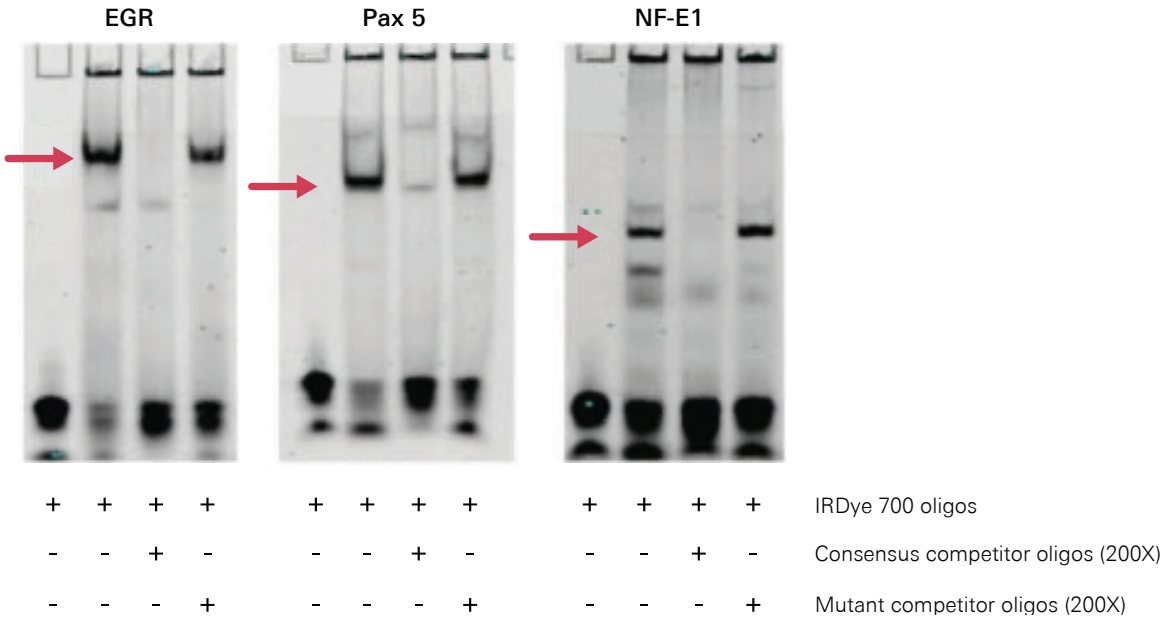


Figure 11. An example EMSA/gel shift assay. IRDye® 700 EMSA performed with consensus oligos for three different transcription factor targets. Arrows indicate positions of mobility shift.

Applications by Odyssey® Imager

	Odyssey XF	Odyssey DLx	Odyssey M
Near-Infrared Fluorescent Western Blot	✓	✓	✓
Visible Fluorescent Western Blot			✓
Chemiluminescent Western Blot	✓		✓*
Colorimetric Western Blot			✓
In-Cell Western™ Assay		✓	✓
On-Cell Western Assay		✓	✓
Cell Health Assay			✓
ELISA			✓
Fluorescent Protein Gel	✓	✓	✓
Colorimetric Protein Gel			✓
Nucleic Acid Gel	✓	✓**	✓
EMSA/Gel Shift Assay		✓	✓
Fluorescent Tissue Section		✓	✓
Histological Staining			✓
Protein Array		✓	✓

**Optional*
***SYTO® 60 only*

Discover the Odyssey® Imager for your applications at

licor.com/odyssey

LI-COR Biotechnology

4647 Superior Street
Lincoln, NE 68504

Phone: +1-402-467-0700
Toll free: 800-645-4267

biosales@licor.com

LI-COR Distributor Network

www.licor.com/bio/distributors

LI-COR Biotechnology GmbH

Siemensstraße 25A
61352 Bad Homburg
Germany

Phone: +49 (0) 6172 17 17 771

bio-eu@licor.com

LI-COR Biotechnology UK Ltd.

St. John's Innovation Centre
Cowley Road
Cambridge
CB4 0WS
United Kingdom

Phone: +44 (0) 1223 422104

bio-eu@licor.com

For patent information, visit www.licor.com/patents.
ISO 9001:2015 certified.

05/22 980-20113