

INFRARED FLUORESCENT PRODUCTS AND APPLICATIONS GUIDE

Advancing Discovery
with Infrared Imaging

LI-COR[®]

Biosciences

35 YEARS OF INNOVATION & EXCELLENCE

IRDye[®] Secondary Antibodies

LI-COR IRDye Infrared Dye Secondary Antibodies and Conjugates are optimized for a wide variety of applications, including:

- Western Blots
- In-Cell Westerns™
- In-Gel Westerns
- On-Cell Westerns
- Protein Arrays
- Tissue Section Imaging
- Molecular Imaging
- Whole Organ Imaging

All IRDye labeled secondary antibodies are highly cross-adsorbed.

| | |
|-----------|--|
| 926-32210 | IRDye 800CW Goat Anti-Mouse IgG (H + L), 0.5 mg |
| 926-32211 | IRDye 800CW Goat Anti-Rabbit IgG (H + L), 0.5 mg |
| 926-32212 | IRDye 800CW Donkey Anti-Mouse IgG (H + L), 0.5 mg |
| 926-32213 | IRDye 800CW Donkey Anti-Rabbit IgG (H + L), 0.5 mg |
| 926-32214 | IRDye 800CW Donkey Anti-Goat IgG(H + L), 0.5 mg |
| 926-32220 | IRDye 680 Goat Anti-Mouse IgG (H + L), 0.5 mg |
| 926-32221 | IRDye 680 Goat Anti-Rabbit IgG (H + L), 0.5 mg |
| 926-32222 | IRDye 680 Donkey Anti-Mouse IgG (H + L), 0.5 mg |
| 926-32223 | IRDye 680 Donkey Anti-Rabbit IgG (H + L), 0.5 mg |

Conjugates

| | |
|-----------|----------------------------------|
| 926-32230 | IRDye 800CW Streptavidin, 0.5 mg |
| 926-32231 | IRDye 680 Streptavidin, 0.5 mg |

Same-day shipping (Federal Express overnight) available on orders shipped to USA if received before 12:00 noon CST. Allow 3-5 days for shipments to Canada. In Germany and U.K., contact LI-COR GmbH. For orders shipped internationally, contact a LI-COR distributor in your area. All shipments subject to availability.

Cost Comparison: Odyssey vs. Chemiluminescence

| Reagents | Odyssey (2 target) | Chemiluminescence 1 target | Chemiluminescence 2 target (strip/reprobe) |
|---------------------------------------|-----------------------|-------------------------------|---|
| Secondary Antibodies | \$0.62 | \$0.94 | \$1.88 |
| Chemiluminescent Substrate (12 ml) | \$0.00 | \$6.05 | \$12.10 |
| Films (2 per membrane) | \$0.00 | \$3.85 | \$7.70 |
| Protein Markers | \$0.22 | \$2.26 | \$2.26 |
| Total Cost* | \$0.84 | \$13.10 | \$23.94 |

* Additional costs such as buffer solutions and membranes are comparable. Prices are representative of typical reagent suppliers. Prices shown in U.S. Dollars.

Western Blotting Kits

Kits With PVDF Membranes

- 926-31050 IRDye Western Kit I
- IRDye 800CW Goat Anti-Mouse Secondary Antibody (25 μ l, 1 mg/ml)
 - IRDye 680 Goat Anti-Rabbit Secondary Antibody (25 μ l, 1 mg/ml)
 - 500 ml Odyssey Blocking Buffer
 - 10 Millipore Immobilon™ -FL PVDF Membranes (0.45 μ m, 10 x 10 cm)
- 926-31052 IRDye Western Kit II
- IRDye 800CW Goat Anti-Rabbit Secondary Antibody (25 μ l, 1 mg/ml)
 - IRDye 680 Goat Anti-Mouse Secondary Antibody (25 μ l, 1 mg/ml)
 - 500 ml Odyssey Blocking Buffer
 - 10 Millipore Immobilon-FL PVDF Membranes (0.45 μ m, 10 x 10 cm)

Kits With Nitrocellulose Membranes

- 926-31058 IRDye Western Kit III
- IRDye 800CW Goat Anti-Mouse Secondary Antibody (25 μ l, 1 mg/ml)
 - IRDye 680 Goat Anti-Rabbit Secondary Antibody (25 μ l, 1 mg/ml)
 - 500 ml Odyssey Blocking Buffer
 - 10 Odyssey Nitrocellulose Membranes (0.22 μ m, 7 x 8.5 cm)
- 926-31060 IRDye Western Kit IV
- IRDye 800CW Goat Anti-Rabbit Secondary Antibody (25 μ l, 1 mg/ml)
 - IRDye 680 Goat Anti-Mouse Secondary Antibody (25 μ l, 1 mg/ml)
 - 500 ml Odyssey Blocking Buffer
 - 10 Odyssey Nitrocellulose Membranes (0.22 μ m, 7 x 8.5 cm)

Membranes and Membrane Kits

- 926-31090 1 package of ten 7 x 8.5 cm Odyssey Nitrocellulose Membranes (0.22 μ m, 7 x 8.5 cm)
- 926-31092 1 roll of Odyssey Nitrocellulose Membrane (0.22 μ m, 30 cm x 3 m roll)
- 829-31080 Blocking Buffer and Membrane Kit
- 10 bottles of Odyssey Blocking Buffer
 - 1 roll of Millipore Immobilon-FL PVDF Membrane (0.45 μ m, 26.5 cm x 3.75 m roll)

In-Cell Western™ Kits

The In-cell Western is an immunocytochemical assay that uses NIR fluorescence to detect and quantify proteins in fixed cells. Detecting proteins in their cellular context increases quantification precision. Proteins in fixed, cultured cells are detected directly in microplates, which yields higher throughput compared to Western blotting and eliminates typical Western blotting steps such as cell lysate preparation, electrophoresis, and membrane transfer.

926-31070 IRDye In-Cell Western Kit I

- IRDye 800CW Goat Anti-Mouse Secondary Antibody (0.5 mg)
 - Odyssey Blocking Buffer, (4 x 500 ml)
 - DRAQ5™ (100 µl)
 - Sapphire700™ Stain (100 µl)
- (Reagents for forty 96-well plates or ten 384-well plates)

926-31072 IRDye In-Cell Western Kit II

- IRDye 800CW Goat Anti-Rabbit Secondary Antibody (0.5 mg)
 - Odyssey Blocking Buffer, (4 x 500 ml)
 - DRAQ5™ (100 µl)
 - Sapphire700™ Stain (100 µl)
- (Reagents for forty 96-well plates or ten 384-well plates)



Phospho-ERK in the 800 channel (green) normalized to DRAQ5+Sapphire700 in the 700 channel (red), eliminating need for a second antibody. Neither DRAQ5 nor Sapphire700 in the IRDye In-Cell Western kits interfere with antibody binding.

Buffers, Reagents, & Accessories

Odyssey Blocking Buffer

LI-COR Odyssey Blocking Buffer is optimized for infrared assays including Westerns and In-Cell Westerns.

- 927-40000 Odyssey Blocking Buffer, 500ml
- 927-40003 Odyssey Blocking Buffer, 500ml, 3 Pack
- 927-40010 Odyssey Blocking Buffer, 500ml, 10 Pack

Electrophoresis and Transfer Buffer

- 928-40010 10X Tris-Glycine Liquid (4 liters) 119.00
- 928-40012 10X Tris-Glycine Dry Pack (2 packs; makes 2 liters total)
- 928-40014 10X Tris-Glycine-SDS Liquid (4 liters)
- 928-40016 10X Tris-Glycine-SDS Dry Pack (2 packs; makes 2 liters)

Wash Buffer

- 928-40018 10X PBS (phosphate-buffered saline) Liquid (4 liters)
- 928-40020 10X PBS Buffer Dry Pack (2 packs; makes 2 liters)

Sample Loading Buffer

- 928-40004 2X Protein Sample Loading Buffer (30 ml)

Protein Stain

- 928-40002 IRDye Blue Protein Stain (1 liter)
Colloidal Coomassie stain for visualizing protein gels in 700 nm channel.
- 928-40022 Sapphire700™ Stain (100 µl)
Cell stain must be used in combination with DRAQ5 for normalization in In-Cell Western™ assays.

Standing orders are offered for some products. Contact LI-COR for details.

Protein Markers

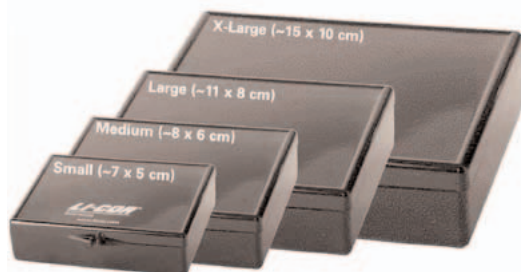
928-40000 Protein Markers, 0.5 ml (10 - 250 kDa, up to 500 lanes)
(Fluoresces in the 700 nm channel)

Western Blot Incubation Boxes

929-97101 Western Incubation Box, Small (1 - 7.3 x 5.1 x 3.0 cm)
 929-97105 Western Incubation Box, Small (five pack)
 929-97110 Western Incubation Box, Small (ten pack)
 929-97201 Western Incubation Box, Medium (1 - 8.9 x 6.6 x 2.9 cm)
 929-97205 Western Incubation Box, Medium (five pack)
 929-97210 Western Incubation Box, Medium (ten pack)
 929-97301 Western Incubation Box, Large (1 - 11.8 x 8.9 x 2.9 cm)
 929-97305 Western Incubation Box, Large (five pack)
 929-97310 Western Incubation Box, Large (ten pack)
 929-97401 Western Incubation Box, X-Large (1 - 15.2 x 10.2 x 3.2 cm)
 929-97405 Western Incubation Box, X-Large (five pack)
 929-97410 Western Incubation Box, X-Large (ten pack)



Odyssey Blocking Buffer
927-40000 (500 ml)



Western Blot Incubation Boxes

IRDye[®] Labeling Reagents

IRDye 800CW Labeling Kits

The IRDye 800CW dye bears an NHS ester reactive group that will couple to free amino groups and form a stable conjugate. Fluorescent conjugates labeled with IRDye 800CW display an absorption maximum of 774 nm and an emission maximum of 789 nm in 1X PBS (see IRDye 800CW graph on page 14).

928-38040 IRDye 800CW Protein Labeling Kit - High Molecular Weight
For labeling 3x 1 mg of protein with molecular weight 45-200 kDa

928-38042 IRDye 800CW Protein Labeling Kit - Low Molecular Weight
For labeling 3x 1 mg of protein with molecular weight 15-45 kDa

928-38044 IRDye 800CW Protein Labeling Kit - Microscale
For labeling 3x 100 µg of protein with molecular weight 14 - 200 kDa

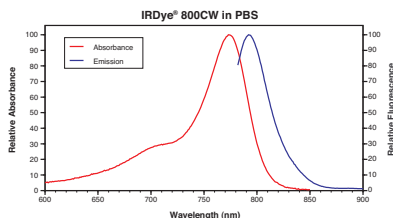
IRDye Near-Infrared Dyes

IRDye 800CW

929-70020 IRDye 800CW NHS Ester 0.5mg

929-70021 IRDye 800CW NHS Ester 5.0mg

IRDye 800CW is the dye of choice for protein/antibody labeling applications and for nucleic acid applications requiring high labeling density. The NHS ester reactive group provides the functionality for labeling primary and secondary amino groups. This dye has the highest water solubility and salt tolerance of the IRDye 800 series.



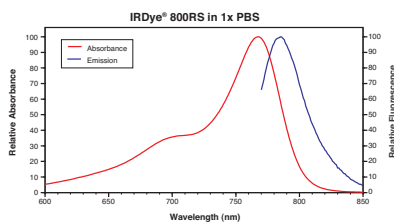
| Solvent | Extinctn. Coeff. M ⁻¹ cm ⁻¹ | Abs Max nm | Em Max nm | Stokes Shift nm | MW g/mole |
|---------------|--|------------|-----------|-----------------|-----------|
| Methanol | 300,000 | 778 | 794 | 16 | 1166 |
| Water | 240,000 | 774 | 789 | 15 | 1166 |
| 1X PBS | 240,000 | 774 | 789 | 15 | 1166 |
| PBS: Methanol | 270,000 | 777 | 791 | 14 | 1166 |

IRDye 800RS

929-72020 IRDye 800RS NHS Ester 0.5mg

929-72021 IRDye 800RS NHS Ester 5.0mg

IRDye 800 RS is the dye of choice for nucleic acid applications. The NHS ester reactive group provides the functionality for labeling primary and secondary amino groups. Nucleic acids labeled with this dye are easily purified with reverse-phase chromatography. The dye has good water solubility, but low salt tolerance, and is more hydrophobic than IRDye 800CW. This dye is not recommended for most protein applications.



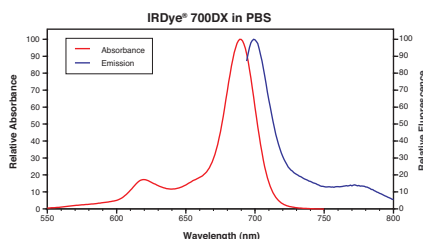
| Solvent | Extinctn. Coeff. $M^{-1} cm^{-1}$ | Abs Max nm | Em Max nm | Stokes Shift nm | MW g/mole |
|---------------|-----------------------------------|------------|-----------|-----------------|-----------|
| Methanol | 300,000 | 770 | 786 | 16 | 961 |
| Water | 200,000 | 767 | 786 | 19 | 961 |
| 1X PBS | 200,000 | 767 | 786 | 19 | 961 |
| PBS: Methanol | 270,000 | 770 | 786 | 16 | 961 |

IRDye 700DX

929-70010 IRDye 700DX NHS Ester 0.5mg

929-70011 IRDye 700DX NHS Ester 5.0mg

IRDye 700 DX is ideal for protein/antibody/nucleic acid labeling and other applications where high water solubility is essential. The NHS ester reactive group provides the functionality for labeling primary and secondary amino groups. This dye has excellent photostability, water solubility, and salt tolerance, but is sensitive to acids.



| Solvent | Extinctn. Coeff. $M^{-1} cm^{-1}$ | Abs Max nm | Em Max nm | Stokes Shift nm | MW g/mole |
|----------|-----------------------------------|------------|-----------|-----------------|-----------|
| Methanol | 210,000 | 680 | 687 | 7 | 1954 |
| Water | 165,000 | 689 | 700 | 11 | 1954 |
| 1X PBS | 165,000 | 689 | 700 | 11 | 1954 |

Molecular Imaging

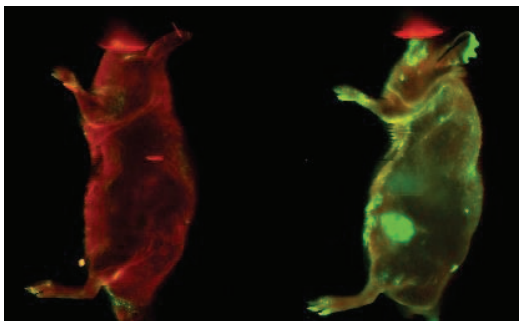
926-08446 IRDye 800CW EGF Optical Probe (10 injections)

IRDye 800CW EGF Optical Probe is a near-infrared (NIR) labeled recombinant human epidermal growth factor (EGF). Epidermal growth factor receptor (EGFR) is one of a family of receptor tyrosine kinases found on the surface of epithelial cells, to which EGF binds.

Many types of cancer cells have abnormally high EGFR levels on the cell surface. *In vivo* assays of EGF binding have proven effective in monitoring specific solid tumor models that exhibit over-expression of EGFR. EGF conjugated to LI-COR's IRDye 800CW NIR fluorophore provides a versatile probe that can be used for *in vitro* and *in vivo* assays, as well as whole organ and tissue section analysis.

Probe Specificity

In-Cell Western™ assays on the LI-COR Odyssey® and Aeries® Infrared Imaging Systems are used to determine binding specificity of the probe *in vitro* for various cell lines. IRDye 800CW EGF has been characterized in A431 (squamous epithelial carcinoma), and PC3M-LN4 and 22Rv1 (human prostate adenocarcinoma) cell lines that over-express EGFR.



Two athymic Nu/Nu tumor-bearing mice were injected with either 0.9% saline (left) or 1 nmol IRDye 800CW EGF (right) 72 hours prior to imaging with the LI-COR Odyssey Infrared Imaging System and Odyssey MousePOD™ imaging accessory.

EMSA Reagents

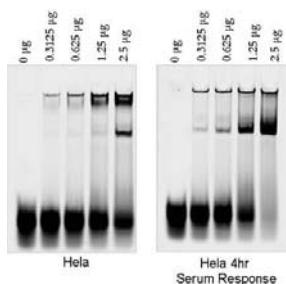
Existing mobility shift assay protocols are easily transformed into infrared assays by replacing oligonucleotides with IRDye end-labeled oligonucleotides. Binding conditions and electrophoresis conditions remain the same as other EMSA detection methods.

Advantages of IRDye labeled DNA detection:

- Linear, 50-fold dilution range from 9.1 fmol to 0.18 fmol
- No hazardous radioisotope, no chemiluminescent substrate reagents, and no film exposure
- Gels are imaged in the glass plates (no gel transfer or drying)

| | |
|-----------|---|
| 927-10100 | 10X Orange Loading Dye |
| 829-07925 | EMSA Oligo, AP-1 (IRDye 700) |
| 829-07933 | EMSA Oligo, ARE (IRDye 700) |
| 829-07923 | EMSA Oligo, CREB (IRDye 700) |
| 829-07927 | EMSA Oligo, E2F (IRDye 700) |
| 829-07928 | EMSA Oligo, EGR (IRDye 700) |
| 829-07929 | EMSA Oligo, HIF-1 (IRDye 700) |
| 829-07924 | EMSA Oligo, NFkB (IRDye 700) |
| 829-07930 | EMSA Oligo, OCT-1 (IRDye 700) |
| 829-07921 | EMSA Oligo, p53 (IRDye 700) |
| 829-07931 | EMSA Oligo, PAX-5 (IRDye 700) |
| 829-07926 | EMSA Oligo, Sp-1 (IRDye 700) |
| 829-07922 | EMSA Oligo, STAT3 (IRDye 700) |
| 829-07932 | EMSA Oligo, YY1/NFE1 (IRDye 700) |
| 4200-31 | Custom Primer, IRDye 700 (1 μ M) (EMSA's require a forward and a reverse primer) |
| 829-07910 | EMSA Buffer Kit (100 reactions) |

Infrared AP-1 EMSA was used to visualize changes in AP-1 binding following three different treatments of HeLa cells. HeLa, HeLa 2 hour serum response (not shown), and HeLa 4 hour serum response nuclear extracts were serially diluted and assayed under the same conditions using IRDye 700 end-labeled oligonucleotide duplex containing AP-1 binding sequence.



Protein Array Accessories



926-30052

Nitrocellulose Coated Slides (25 pack)

Offers greater binding capacity than other matrices and excellent signal-to-noise with infrared detection.



926-30062

Glass Slide Array Replicator

Used with the glass slide indexing system to place 768 spots (~ 500 microns) in a 18 x 36 mm grid on two separate slides.



926-30060

Glass Slide Indexing System

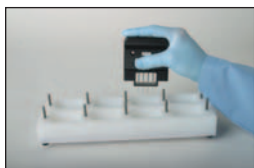
Spring-loaded locator stabilizes 25 x 75 mm glass slides (1 mm thick).



926-30064

Microplate Indexing System

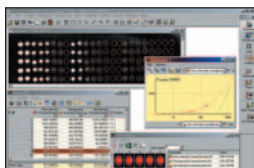
Registers replicator pins to the center of microplate wells for consistent spot diameters.



926-30066

Wash and Blot Station

Washes and cleans replicator pins between spotting.



926-30050

ArrayPro® Analyzer Software

926-30068 Lint Free Blotting Paper

926-30070 Pin Cleaning Solution

926-30072 Replacement Pins

Ordering Information

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