GE Healthcare

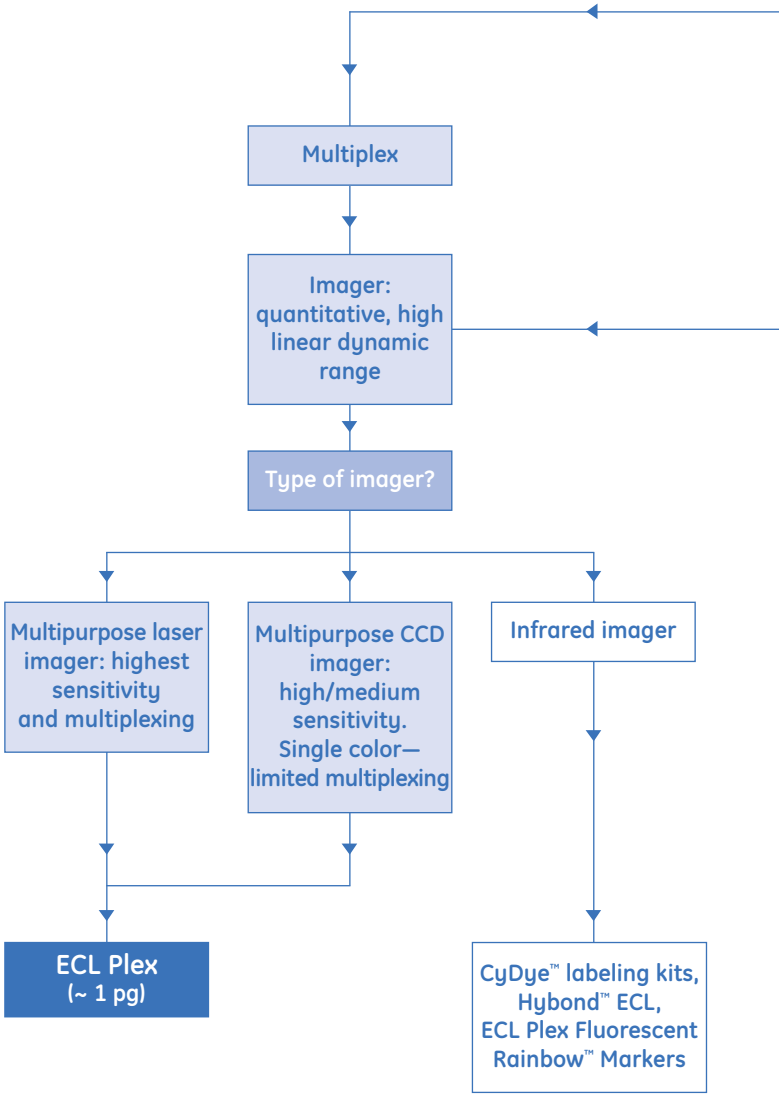
Powerful solutions for Western blotting

Reach new heights in performance and versatility
ECL • ECL Plus • ECL Advance • ECL Plex

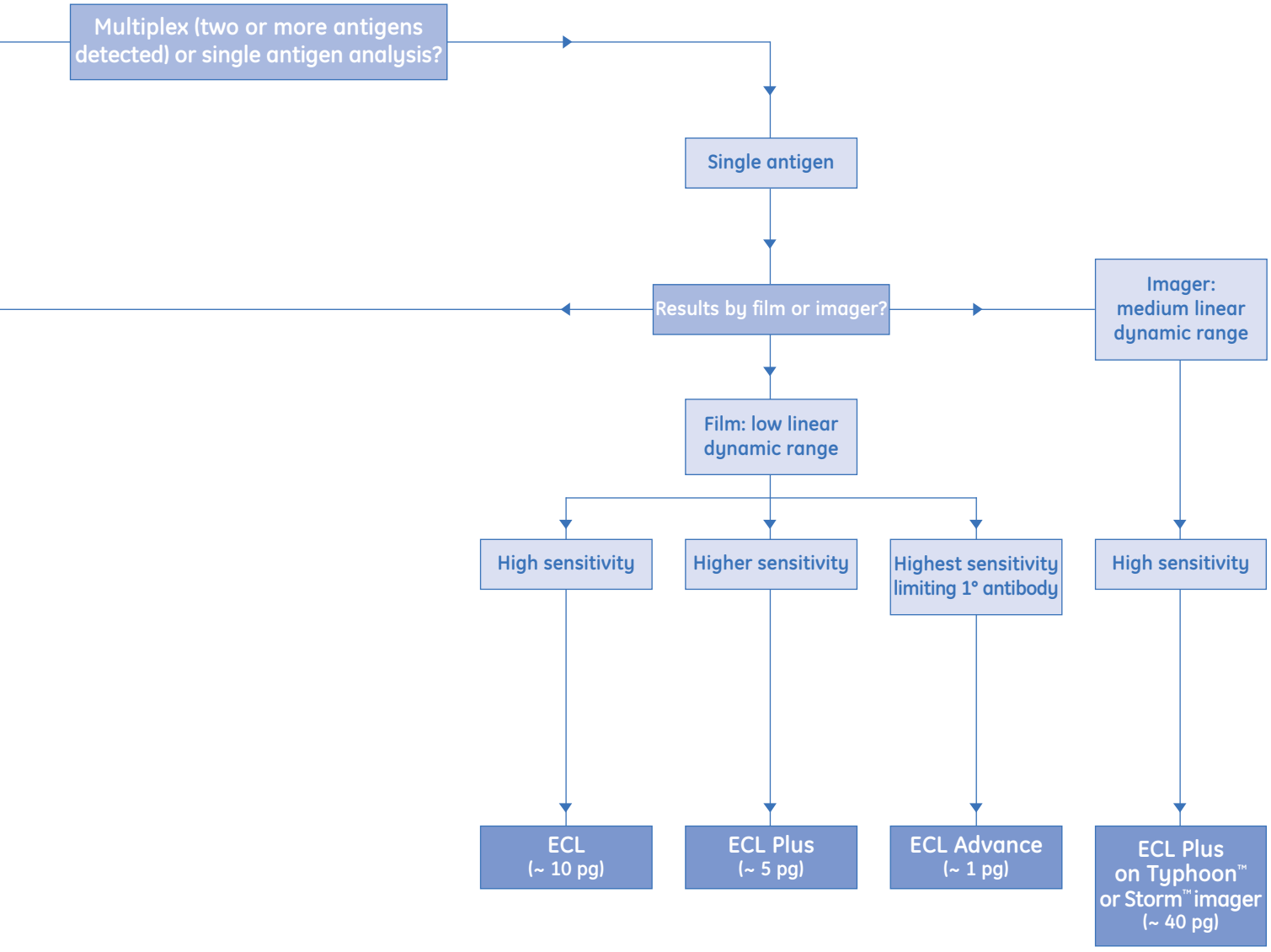
Powerful solutions from your single source

ECL Western Blotting Systems from GE Healthcare have been delivering sensitivity, speed, and versatility for over 15 years now. Today, the world's first chemiluminescent reagents continue to be the most widely used and have established a well-earned reputation for excellence you can rely on, day in, day out. ECL™, ECL Plus™, and ECL Advance™ are now complemented by the latest addition to the innovative range of ECL Western Blotting Systems from GE Healthcare, the new ECL Plex™ fluorescent system, expanding your options even further.

No matter what your Western blotting requirements are, GE Healthcare offers a choice of sensitivities and protocols ideally suited to meet the challenges presented by Western blotting experiments. The continuing evolution of ECL Western Blotting Systems ensures that you can always select the solution best suited to your particular application.



Selection guide for ECL Western Blotting Systems



The power of ECL Systems

ECL Plex—multiplex detection on multipurpose imagers

- Optimized system for quantitative analysis
- CyDye fluorescent technology enables multiwavelength detection
- No need to strip and reprobe blots, reducing variability
- Measures significant differences in protein levels with high specificity
- Highest sensitivity with multiplexing (~ 1 pg in model system)

ECL Advance—minimizes the use of valuable antibody

- Primary antibody concentrations as low as 1 : 100 000
- Maximum signal-to-noise ratio
- Demonstrably superior to other high-sensitivity reagents
- Highest sensitivity with minimum amount of antibody (~ 1 pg in model system, equivalent to ECL Plex)

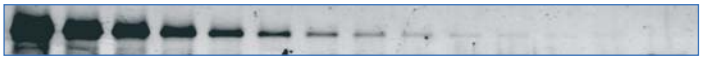
ECL Plus—chemiluminescent and chemifluorescent detection

- Higher sensitivity (~ 5 pg in model system using film, ~ 40 pg using imager)
- Intense light output at 440 nm
- Extended signal duration
- Quantitative detection on a fluorescent scanner

ECL—world's first chemiluminescent reagent for Western blotting

- Excellent reagent for high-sensitivity detection
- 10-fold more sensitive than colorimetric methods
- High sensitivity (~ 10 pg in model system)

Comparison of ECL Plex, ECL Advance, ECL Plus, and ECL in a model system using recommended protocols with optimized antibody concentrations

	Primary antibody	Secondary antibody	Linearity (R^2)	Dynamic range*	Sensitivity (pg)
ECL Plex 	1 : 750	1 : 2 500	0.998	3.6	~ 1
ECL Advance 	1 : 100 000	1 : 400 000	0.978	2.4	~ 1
ECL Plus 	1 : 10 000	1 : 100 000	0.997	2.4	~ 5
ECL 	1 : 1 500	1 : 10 000	0.961	1.5	~ 10

Target: human apotransferrin two-fold dilutions starting at 5 ng. Detection: ECL Plex, Typhoon 9410; all others, Hyperfilm™ ECL.
Primary antibody: rabbit polyclonal anti–human transferrin. Secondary antibody: ECL Plex, ECL Plex goat-α-rabbit-Cy5; all others, donkey-α-rabbit IgG (HRP-linked).
ECL Plus chemifluorescent detection: linearity, $R^2 = 0.994$; dynamic range = 2.1; sensitivity = 39 pg.

*Orders of magnitude

Highest dynamic range and linearity

ECL Plex

Sensitivity

ECL Plex = **ECL Advance** > **ECL Plus** > **ECL**

NEW

ECL Plex

Western Blotting Detection System

Quantitative analysis and multiplex detection on multipurpose imagers

A major advantage of the new ECL Plex System is the use of two fluorescent dye labels (Cy3, λ_{max} 570 nm, and Cy5, λ_{max} 670 nm), which enables you to carry out simultaneous two-color protein analysis on Western blots. This benefit is not available on systems using chemiluminescent detection. The result is faster, more accurate differential analysis and a reduction in the variability caused by stripping and reprobing blots.

By delivering superior image clarity and direct fluorescence detection (without enzyme-substrate amplification), the ECL Plex System provides you with increased reproducibility and accuracy for the measurement of protein levels. ECL Plex is ideally suited for use with the Typhoon multipurpose imager and is fully compatible with Hybond ECL (nitrocellulose) and the new Hybond-LFP (low-fluorescent PVDF) membrane.

Optimized for use with Typhoon and other multipurpose imagers*

- No extra cost for additional capital equipment for limited applications
- No requirement to learn new systems and software
- Designed for use with existing and future imaging systems
- Rapid scanning time for multiple blots

Highest sensitivity with multiplexing (~ 1 pg in model system)

- Proven CyDye™ technology enables multiwavelength detection
- No need to strip and reprobe blots when using two antibodies, reducing variability

Quantitative with broadest dynamic range and highest linearity

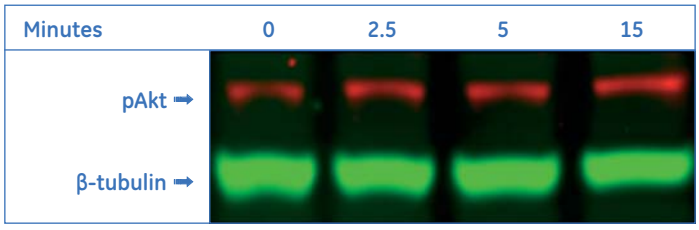
- Highly accurate measurement of significant differences in protein levels
- Long-lasting signal allows stored blots to be rescanned months later

Complete system to provide maximum specificity

- High-performance, validated system
- Delivers increased reproducibility and accuracy
- Minimum cross-reactivity with maximum signal-to-noise ratio

* Tested on Typhoon 9410, Ettan™ DIGE Imager, Storm 860, and a range of other imagers capable of detecting Cy3 and Cy5 fluorescence (data available on request).

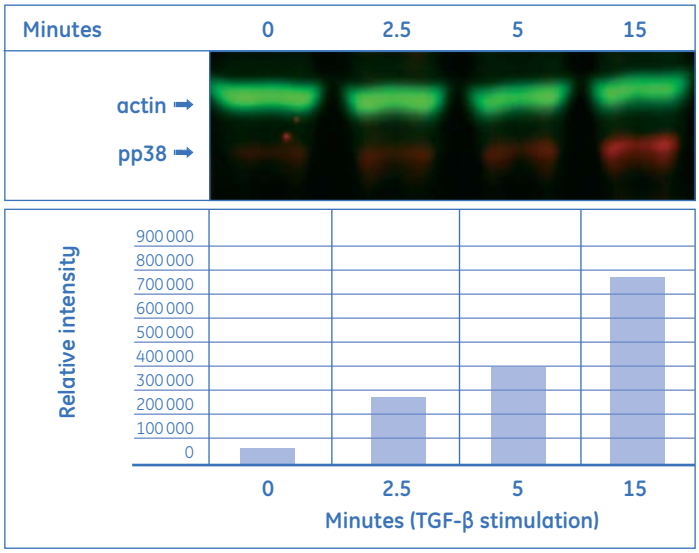
Multiplexing with highest sensitivity



Simultaneous detection of the low-abundant protein phospho-Akt (pAkt) with β-tubulin control

Targets: phospho-Akt (pAkt) and human β-tubulin in T293 cells stimulated 0, 2.5, 5, or 15 min with TGF-β, phosphorylated and total protein detected.
Detection: Hybond ECL on Typhoon 9410.
Primary antibodies: polyclonal phospho-Akt (Ser 473) and monoclonal anti-β-tubulin.
Secondary antibody: ECL Plex goat-α-rabbit IgG-Cy5 + ECL Plex goat-α-mouse IgG-Cy3.

Quantitative



Relative quantitation of the activation of phosphorylated p38 (pp38)

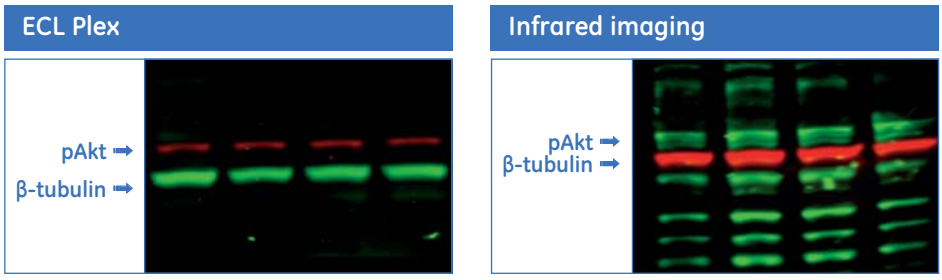
Targets: phosphorylated p38 (pp38) and human actin in T293 cells stimulated 0, 2.5, 5, or 15 min with TGF-β, phosphorylated and total protein detected.
Detection: Hybond-LFP on Typhoon 9410.
Primary antibodies: polyclonal anti-phospho-p38 MAP kinase (Thr 180/Tyr 182) and monoclonal anti-actin.
Secondary antibodies: ECL Plex goat-α-rabbit IgG-Cy5 + ECL Plex goat-α-mouse IgG-Cy3.

The relative intensity is the scored intensity value of pp38 when quantitated relative to the total amount of anti-actin.

ECL Plex

Western Blotting Detection System

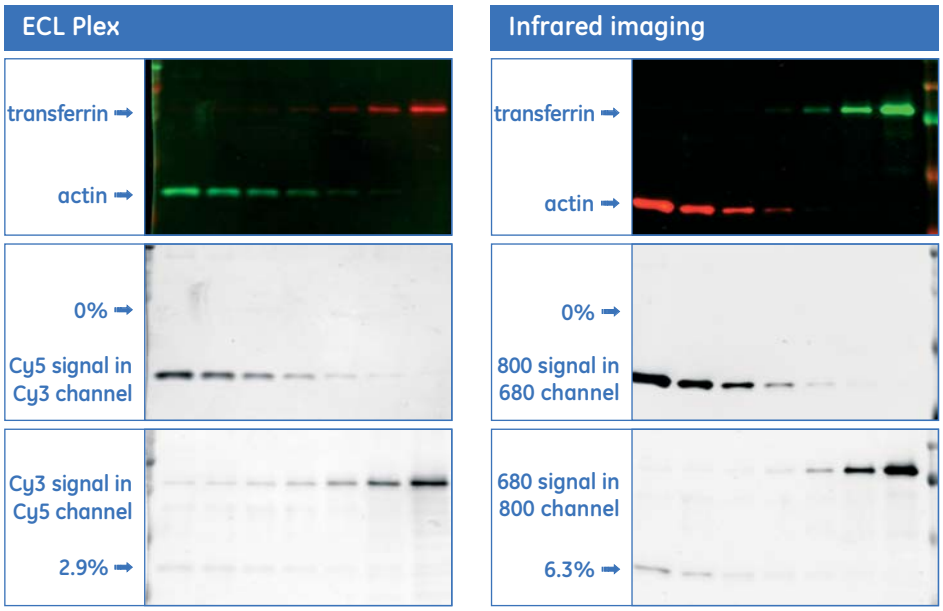
Optimized ECL Plex System has the highest specificity...



Specific detection of phospho-Akt (pAkt) and β-tubulin

Targets: phospho-Akt (pAkt) and human β-tubulin in T293 cells stimulated 0, 2.5, 5, or 15 min with TGF-β, phosphorylated and total protein detected.
Detection: Hybond ECL on Typhoon 9410 (left); LI-COR Odyssey™ scanner (right), used according to manufacturer's recommended protocols with reagents from Invitrogen and Rockland Immunochemicals Inc.*
Primary antibodies: polyclonal anti-phospho-Akt (Ser 473) and monoclonal anti-β-tubulin.
Secondary antibodies: ECL Plex goat-α-rabbit IgG-Cy5 and ECL Plex goat-α-mouse IgG-Cy3 (left); Alexa Fluor™ 680 goat-α-mouse IgG and IRDye™ 800 goat-α-rabbit IgG (right).

...and the lowest cross-reactivity



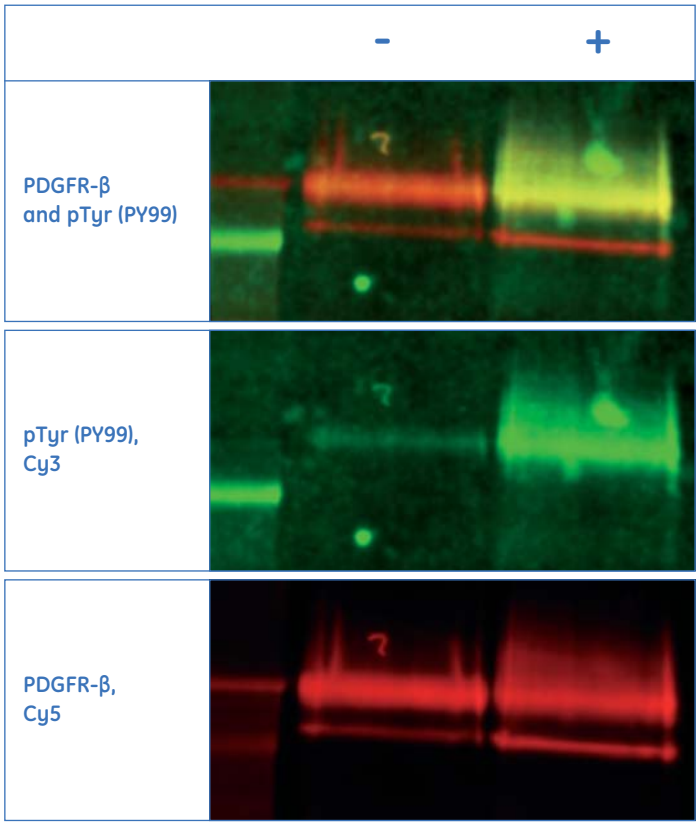
Lower false signals from dye interactions

Targets: human apotransferrin four-fold dilutions starting at 5 ng, bovine cardiac muscle actin two-fold dilutions starting at 150 ng.
Detection: Hybond ECL on Typhoon 9410 (left); LI-COR Odyssey scanner (right), used according to manufacturer's recommended protocols with reagents from Invitrogen and Rockland Immunochemicals Inc.*
Primary antibodies: monoclonal anti-actin and rabbit polyclonal anti–human transferrin.
Secondary antibodies: ECL Plex goat-α-rabbit IgG-Cy5 and ECL Plex goat-α-mouse IgG-Cy3 (left); Alexa Fluor 680 goat-α-mouse IgG and IRDye 800 goat-α-rabbit IgG (right).

False signals in respective channels are shown (%).

*For more information, please see our ECL Plex application note 28-4015-40 AA

Proven in experimental systems



PDGF-BB stimulation (+) of PAE cells transfected with PDGFR-β leads to PY99 kinase activation through binding of PDGF-BB to the expressed receptor

Data courtesy of Dr. Johan Lennartsson, Ludwig Institute, Uppsala, Sweden

Targets: tyrosine phosphorylation (PY99) and PDGFR-β in porcine aortic endothelial (PAE) cells. Detection: Hybond ECL on Typhoon 9400.
Primary antibodies: rabbit polyclonal anti-PDGFR-β and mouse monoclonal anti-phosphotyrosine (PY99).
Secondary antibodies: ECL Plex goat-α-rabbit IgG-Cy5 and ECL Plex goat-α-mouse IgG-Cy3.

ECL Advance

Western Blotting Detection System

Achieve highest sensitivity with the minimum amount of valuable antibody

The ECL Advance Western Blotting Detection System is designed to provide greater sensitivity in experiments involving a low concentration of the protein of interest or limited primary antibody. The intense light output provided by ECL Advance enables significantly reduced antibody concentrations to be used.

The system contains ECL Advance—a proprietary, high-performance HRP chemiluminescent substrate—and the ECL Advance Blocking Agent, which effectively controls background while retaining the high level of signal intensity. These features combine to give you the highest sensitivity (ECL Advance is up to 10 times more sensitive than ECL Plus) with the lowest amount of antibody.

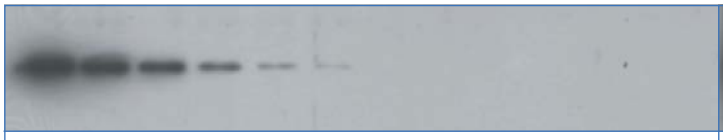
- Highest sensitivity with minimum amount of antibody (~ 1 pg in model system, equivalent to ECL Plex)
- Significantly reduced antibody concentrations (up to one million-fold 2° antibody dilutions have been used)
- Long-lasting light output
- Compatible with Hybond ECL and Hybond-P membranes (Hybond-P is more robust for stripping and reprobing)
- Maximized signal-to-noise ratio with ECL Advance Blocking Agent
- Detectable on Hyperfilm ECL or CCD imager

ECL Advance: highest-sensitivity chemiluminescent system, requiring very low amounts of primary and secondary antibodies

ECL Advance	Roche Lumi-Light PLUS
	
Sensitivity 0.6 pg	19.5 pg
Primary antibody 1 : 100 000	1 : 10 000
Secondary antibody 1 : 400 000	1 : 100 000
Membrane Hybond ECL	Hybond ECL

Target: human apotransferrin two-fold dilutions starting at 5 ng. Detection: Hyperfilm ECL.
Primary antibody: rabbit polyclonal anti–human transferrin. Secondary antibody: HRP-anti-rabbit IgG.

ECL Advance has much higher sensitivity than Roche Lumi-Light™ PLUS at $^1/_{10}$ the primary antibody and $^1/_4$ the secondary antibody concentrations.

ECL Advance	Pierce SuperSignal West Femto Maximum Sensitivity Substrate
	
Primary antibody 1 : 50 000	1 : 5 000
Secondary antibody 1 : 200 000	1 : 100 000
Membrane Hybond ECL	(manufacturer's recommended conditions)

Target: bovine actin two-fold dilutions starting at 50 ng. Detection: Hyperfilm ECL (5-min exposure).
Primary antibody: monoclonal anti-actin. Secondary antibody: HRP-anti-mouse IgG.

Even when 10 times less primary antibody and half as much secondary antibody are used, ECL Advance is still more sensitive than Pierce SuperSignal™ West Femto Maximum Sensitivity Substrate.

ECL Plus

Western Blotting Detection System

The only chemiluminescent and chemifluorescent solution for Western blotting

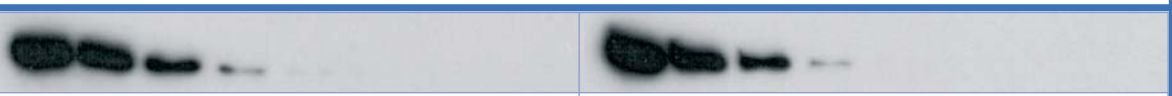
ECL Plus Western Blotting Detection Systems deliver superior sensitivity with the added advantage of chemifluorescent detection on an appropriate scanner. This versatile functionality enables you to carry out qualitative analysis (on film) and semiquantitative analysis using CCD imagers or laser scanners with single antibody or stripping and reprobing.

The acridan-based substrate used in ECL Plus Western Blotting Detection Systems releases a high level sustained output of light and, in addition, generates an intense chemifluorescent signal at 440 nm. This chemifluorescent signal can be detected on a fluorescence imager with the appropriate filters.

- Higher sensitivity (~ 5 pg in model system using film, ~ 40 pg using imager)
- Chemiluminescent and chemifluorescent detection
- Extended signal duration (up to 48 hours) enables multiple exposures to be made
- Compatibility with Hybond ECL and Hybond-P membranes (Hybond-P is more robust for stripping and reprobing)

ECL Plus: higher-sensitivity chemiluminescent system with long-lasting signal

1 min exposure

	ECL Plus	Pierce SuperSignal West Dura
		
Primary antibody	1:1000	1:1000
Secondary antibody	1:50000	1:100000
Detection	Hyperfilm ECL, 1 min exposure	Hyperfilm ECL, 1 min exposure

24 h later

	ECL Plus	Pierce SuperSignal West Dura
		
Primary antibody	1:1000	1:1000
Secondary antibody	1:50000	1:100000
Detection - Hyperfilm ECL, 2 h exposure, 24 h after substrate application		

Target: actin two-fold dilutions starting at 100 ng.

ECL

Western Blotting Detection System

The world's first chemiluminescent reagent for Western blotting

ECL Western Blotting Detection Systems were the world's first commercial chemiluminescent reagents for Western blotting and are still the most widely used. They are at least 10-fold more sensitive than colorimetric assays and produce excellent and reliable results every time.

These exceptional reagents for high-sensitivity detection use enhanced luminol-based detection which is suitable for all routine Western blotting. ECL Western Blotting Detection Systems, optimized with Hybond ECL Membranes, form a complete solution.

- Proven performance
- Most frequently cited in publications
- High sensitivity (~ 10 pg in model system)
- Detectable on Hyperfilm ECL or CCD imager

Ordering information

ECL Western Blotting Detection Systems

ECL Plex

ECL Plex Western Blotting Combination Packs

Sufficient for at least 1 000 cm^2 of membrane

Cy3, Cy5, Hybond ECL

ECL Plex Western Blotting Combination Pack RPN998

Combination pack optimized for ECL Plex Western Blotting System, including Hybond ECL (nitrocellulose membrane). Contains the following components, sufficient for at least 1 000 cm^2 of membrane:

Pack contents	Qty / Size	Code No.
ECL Plex goat-α-mouse IgG-Cy3	150 µg	PA43009
ECL Plex goat-α-rabbit IgG-Cy5	150 µg	PA45011
ECL Plex Fluorescent Rainbow Markers	120 µl	RPN850
Hybond ECL	10 × 10 cm, 10 sheets	RPN1010D
Protocol		

Cy3, Cy5, Hybond-LFP

ECL Plex Western Blotting Combination Pack RPN999

Combination pack optimized for ECL Plex Western Blotting System, including Hybond LFP (low-fluorescent PVDF membrane). Contains the following components, sufficient for at least 1 000cm^2 of membrane:

Pack contents	Qty / Size	Code No.
ECL Plex goat-α-mouse IgG-Cy3	150 µg	PA43009
ECL Plex goat-α-rabbit IgG-Cy5	150 µg	PA45011
ECL Plex Fluorescent Rainbow Markers	120 µl	RPN850
Hybond-LFP	20 × 20 cm, 3 sheets	RPN2020LFP3
Protocol		

ECL Plex Western Blotting Detection Reagents

Optimized for use with the ECL Plex system

ECL Plex CyDye-conjugated antibodies

Product	Qty	Membrane area, at least…	Code No.
ECL Plex goat-α-mouse IgG-Cy3	150 µg	1 000 cm^2	PA43009
	600 µg	4 000 cm^2	PA43010
ECL Plex goat-α-mouse IgG-Cy5	150 µg	1 000 cm^2	PA45009
	600 µg	4 000 cm^2	PA45010
ECL Plex goat-α-rabbit IgG-Cy5	150 µg	1 000 cm^2	PA45011
	600 µg	4 000 cm^2	PA45012

Hybond-LFP

Low-fluorescent PVDF membrane, 0.2-µm pore size.

Product	Qty	Size	Code No.
Hybond-LFP	3 sheets	20 × 20 cm	RPN2020LFP3
	10 sheets	20 × 20 cm	RPN2020LFP
	15 sheets	14 × 16 cm	RPN1416LFP
	1 roll	30 cm × 3m	RPN303LFP

ECL Plex Fluorescent Rainbow Markers

Full-range, defined molecular weight standards from 10 to 250 kDa. Supplied in gel loading buffer.

Product	Qty	Code No.
ECL Plex Fluorescent Rainbow Markers	120 µl	RPN850
	500 µl	RPN851

Blocking Buffer

Product	Qty	Code No.
BSA	25 g	RPN412

ECL Advance

Product	Membrane area, at least…	Code No.
ECL Advance Western Blotting Detection Kit	1 000 cm^2	RPN2135

System contents

ECL Advance Solution A
ECL Advance Solution B
ECL Advance Blocking Agent
Protocol

ECL Plus

Product	Membrane area, at least…	Code No.
ECL Plus Western Blotting Detection Reagents	1 000 cm^2	RPN2132
	3 000 cm^2	RPN2133

System contents

Solution A
Solution B
Protocol

Product	Number of miniblots	Code No.
ECL Plus Western Blotting Reagent Pack	10 (10 × 10 cm)	RPN2124

Pack contents

Anti-mouse IgG HRP conjugate
Anti-rabbit IgG HRP conjugate
ECL membrane-blocking agent
Protocol

ECL

Product	Membrane area, at least…	Code No.
ECL Western Blotting Detection Reagents	6 000 cm^2	RPN2134
	4 000 cm^2	RPN2106
	2 000 cm^2	RPN2209
	1 000 cm^2	RPN2109

System contents

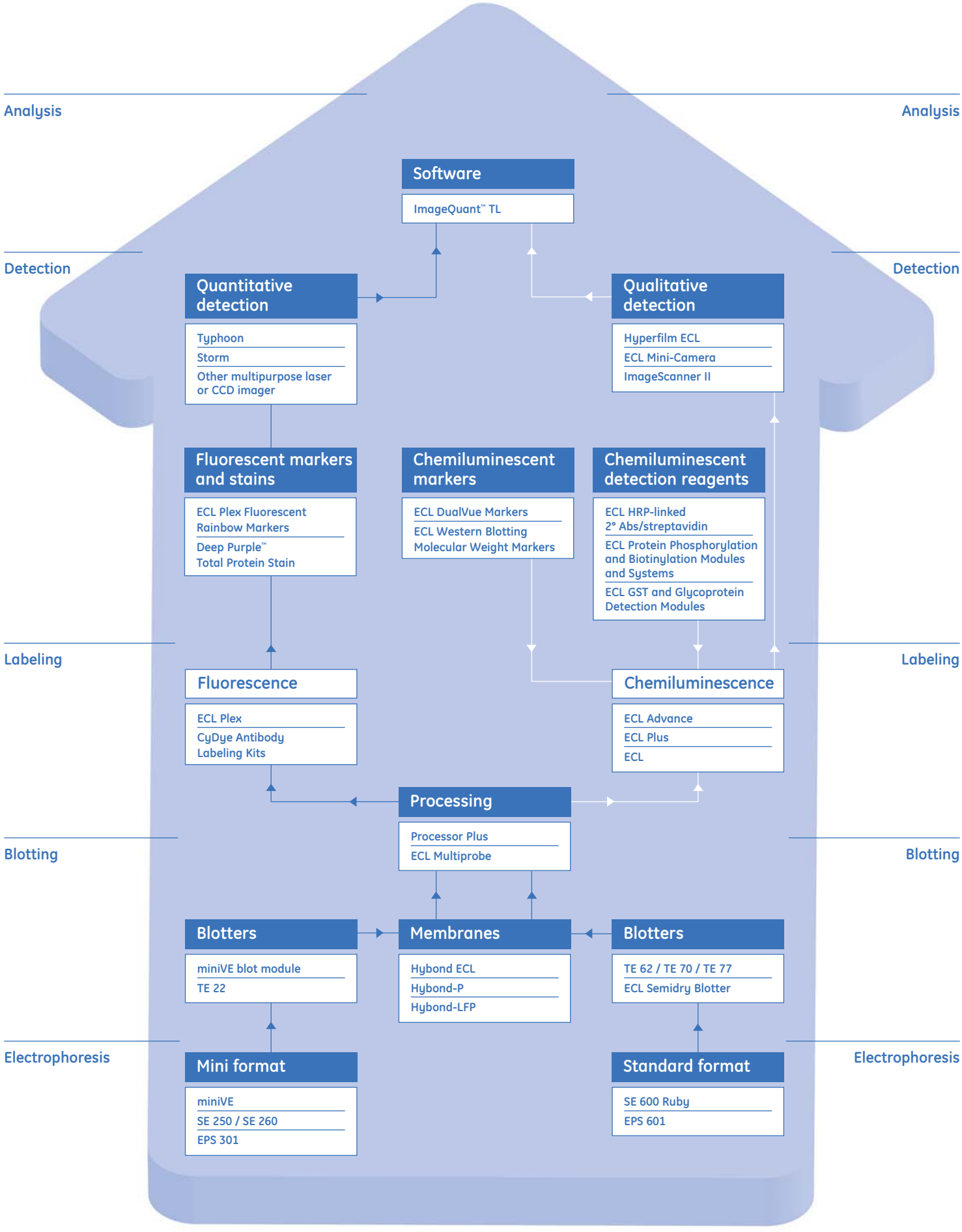
Detection Reagent 1
Detection Reagent 2
Protocol

Product	Number of miniblots	Code No.
ECL Western Blotting System	10 (10 × 10 cm)	RPN2108

System contents

Detection Reagent 1
Detection Reagent 2
Sheep anti-mouse IgG (HRP-linked)
Donkey anti-rabbit IgG (HRP-linked)
Blocking reagent
Protocol

Related products



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ECL Plus Western blotting detection reagents are manufactured for GE Healthcare by Lumigen Inc. This component is covered by US patent numbers 5,491,072 and 5,593,845 and foreign equivalents and is sold under license from Lumigen Inc.

Bulk CyDyes: Notice to purchaser about limited license: The purchase of Mono and Bis dyes, mono esters, maleimides, and hydrazides includes a limited non-exclusive sublicense under US patent number 5,268,486 and 4,981,977 to use them solely for non-commercial research for which no fee from third parties are being derived. There is no implied license hereunder for any commercial use.

CyDye or portions thereof is manufactured under a license from Carnegie Mellon University under patent number 5,268,486 and other patents pending. The purchase of CyDye DIGE Fluors includes a limited license to use the CyDye Fluors for internal research and development, but not for any commercial purposes. A license to use the CyDye Fluors for commercial purposes is subject to a separate license agreement with GE Healthcare Limited.

Deep Purple Total Protein Stain is exclusively licensed to Amersham Biosciences from Fluorotechnics Pty Ltd. Deep Purple Total Protein Stain may only be used for applications in life science research.

Amersham Biosciences Corp., a General Electric Company going to market as GE Healthcare.

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14-0003-87 AB 12/2005 Printed in Germany