Amersham Biosciences
Part of GE Healthcare

ECL Plex Western Blotting Detection System

Multiplex protein detection based on direct fluorescent CyDye-labeled conjugates

With the ECL Plex™ Western Blotting Detection System, GE Healthcare introduces a number of new products under the well-established ECL™ brand (ECL, ECL Plus™, and ECL Advance™). ECL Plex uses direct fluorescent light detection in contrast to the earlier products, which are based on detection of chemiluminescent or chemifluorescent signals. The ECL Plex system reaches a limit of detection of 1.2 pg in a model system, with a dynamic range over 3.6 orders of magnitude. In the multiplex application, two proteins can be detected in the same blot with minimal cross-reactivity between antibodies or dyes.

ECL Plex offers:

- Compatibility with Typhoon™ and other multipurpose imagers.* No requirement for additional capital equipment.
- Multiplex analysis with high sensitivity. Proven CyDye™ technology enables multiwavelength detection. No need to strip and reprobe blots—avoids protein loss and saves time.
- Quantitative analysis with broadest dynamic range and highest linearity—detects significant differences in protein levels with high accuracy. Rescanning of membranes is possible after months.
- Optimized system giving the highest specificity—increased accuracy of results.
- Simple protocol for fast analysis with nontoxic products.

Western blotting is an important tool in protein analysis. Current techniques based on enhanced chemiluminescence are very sensitive but offer limited dynamic range and accuracy of quantitation. Fluorescent Western blotting, on the other hand, can have problems with high background from membranes and cross-talk (spectral overlap) between dyes. These issues have now been addressed by the ECL Plex system.

The ECL Plex system consists of products selected and optimized for best performance regarding sensitivity, dynamic range, linearity, and signal-to-noise ratio. Together with a high-performance multipurpose imager, such as the Typhoon scanner, this provides high quality data for single or multiplex (Fig 1) analyses.

It is also possible to perform direct in-gel detection, without blotting onto a membrane. However, this is only recommended for highly expressed proteins and is not quantitative.

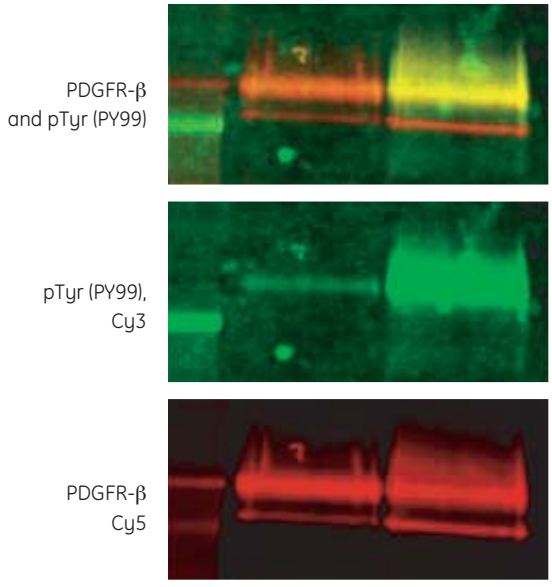


Fig 1. PDGF-BB stimulation (+) of porcine aortic endothelial (PAE) cells transfected with PDGFR-β leads to tyrosine phosphorylation of the receptor. Detection with mouse monoclonal anti-phosphotyrosine (PY99). Total receptor levels detected with rabbit polyclonal anti–PDGFR-β. *Data courtesy of Dr. Johan Lennartsson, Ludwig Institute, Uppsala, Sweden.*

* Tested on Typhoon 9410, Ettan™ DIGE Imager, Storm™ 860, and a range of other imagers capable of detecting Cy™3 and Cy5 fluorescence (data available on request).

High sensitivity and linearity with wide dynamic range

Optimization of conjugated antibodies, membranes with low fluorescence characteristics, and blocking buffer provides high-quality results. Three different ECL Plex secondary antibody conjugates have been developed for best performance. The following membranes have been selected: Hybond™ ECL, low-fluorescent nitrocellulose, and Hybond-LFP, a new low-fluorescent PVDF membrane recommended when stripping is required. Many blocking solutions are compatible (Table 1), but we recommend 5% bovine serum albumin (BSA) in PBS for most applications. To reduce nonspecific detection, we recommend the use of 2% ECL Advance Blocking Agent in PBS-T (Table 1).

Table 1. Compatibility of blocking solutions with the ECL Plex system.

Membrane	5% ECL Blocking Agent in PBS-T	2% ECL Advance Blocking Agent in PBS-T	5 % BSA in PBS	10% gelatin	PBS-T
Hybond ECL	++	+++	+++	–	++
Hybond-LFP	++	++++	+++	–	++

++++ = high performance
+++ = good performance
++ = acceptable performance
+ = poor performance
– = not compatible

Ratings are based on overall performance, including level of autofluorescence/background, nonspecific detection, and signal intensity.

Figures 2–4 show performance of the respective ECL Plex secondary antibodies on both types of membranes.

Detection of multiple protein targets

Secondary antibodies labeled with different CyDyes allow detection of fluorescent signal at different wavelengths. This allows multiple proteins to be analyzed on the same blot without stripping and reprobing the membrane. The optimized ECL Plex secondary antibody conjugates ensure lowest cross-reactivity and highest confidence for quantitation. Figure 5 shows dual protein detection using an optimized antibody pair.

Reduced nonspecific detection

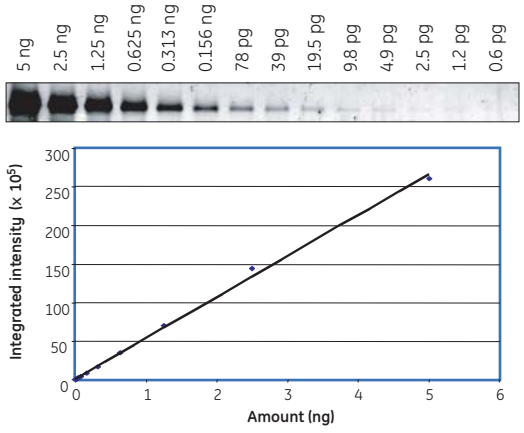
Background noise levels can vary between samples depending on the detection system used. ECL Plex complements the chemiluminescent kits from GE Healthcare and can reduce nonspecific detection. Detection of SMAD3 protein was performed in lysates from different cell lines using the ECL Advance chemiluminescent kit and the ECL Plex kit (Fig 6).

ECL Plex technical data

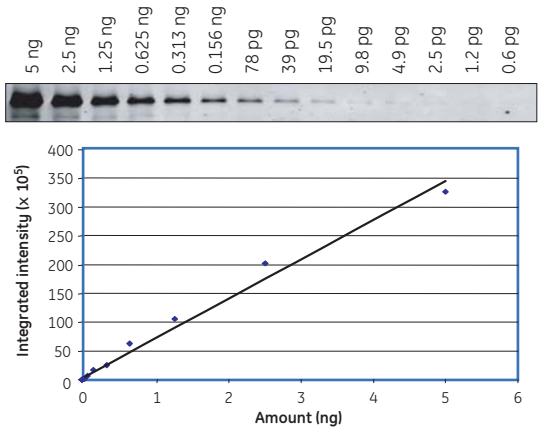
		λ_{max} (nm)	
		Excitation	Emission
CyDye characteristics	Cy3	550	570
	Cy5	649	670

Sensitivity	1.2 pg in model system*
Primary antibody dilution range	1:100–1:5000
ECL Plex secondary antibody conjugates dilution range	1:1250–1:4000
Emission duration (on membrane)	>3 months, protected from light
Recommended membrane	Hybond ECL or Hybond-LFP
Recommended detection method	Fluorescence imager compatible with Cy3 and Cy5 dyes
Recommended use	High sensitivity, multiplexing, linear quantitation

*(in the same model system, ECL Advance shows similar sensitivity; ECL Plus detects ~5 pg; and ECL detects ~10 pg)

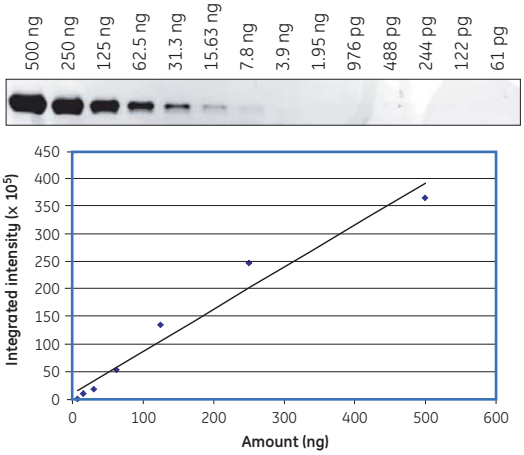


Linearity, Hybond ECL **DR:** 5 ng–1.2 pg **R^2:** 0.998

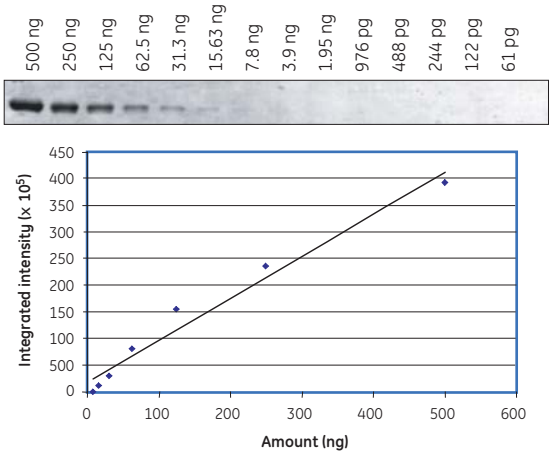


Linearity, Hybond-LFP (0.2 μm) **DR:** 5 ng–1.2 pg **R^2:** 0.986

Fig 2. ECL Plex single protein detection on Hybond ECL (left) and Hybond-LFP (right): human apotransferrin, 5 ng–0.6 pg (two-fold dilutions). Primary antibody: rabbit polyclonal anti–human transferrin; secondary antibody: ECL Plex goat-α-rabbit IgG-Cy5. (Dynamic range = DR; linearity = R^2).

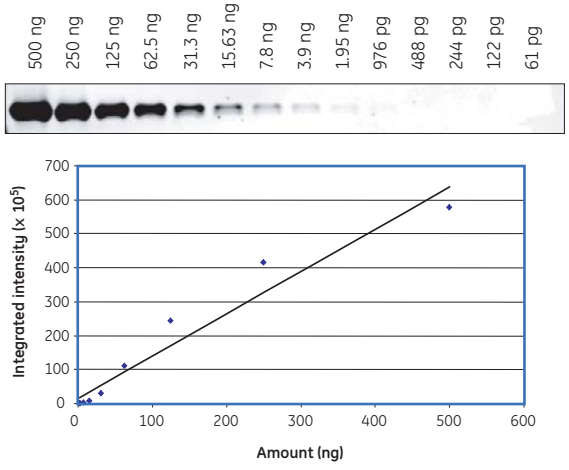


Linearity, Hybond ECL **DR:** 500–7.81 ng **R^2:** 0.963

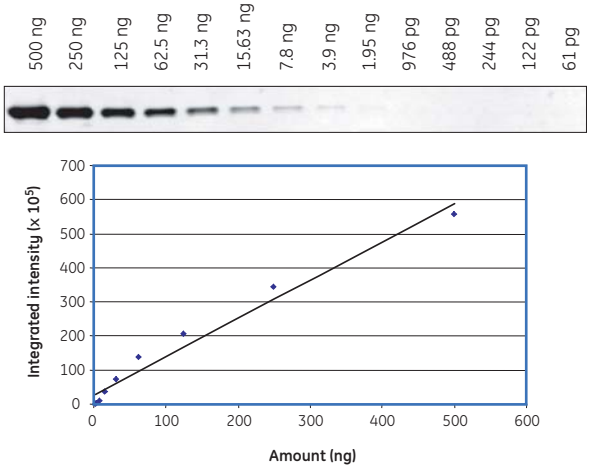


Linearity, Hybond-LFP (0.2 μm) **DR:** 500–7.81 ng **R^2:** 0.971

Fig 3. ECL Plex single protein detection on Hybond ECL (left) and Hybond-LFP (right): bovine cardiac muscle actin, 500 ng–61 pg (two-fold dilutions). Primary antibody: monoclonal anti-actin; secondary antibody: ECL Plex goat-α-mouse IgG-Cy3. (Dynamic range = DR; linearity = R^2).



Linearity, Hybond ECL **DR:** 500–1.95 ng **R^2:** 0.947



Linearity, Hybond-LFP (0.2 μm) **DR:** 500–1.95 ng **R^2:** 0.973

Fig 4. ECL Plex single protein detection on Hybond ECL (left) and Hybond-LFP (right): bovine cardiac muscle actin, 500 ng–61 pg (two-fold dilutions). Primary antibody: monoclonal anti-actin; secondary antibody: ECL Plex goat-α-mouse IgG-Cy5). (Dynamic range = DR; linearity = R^2).

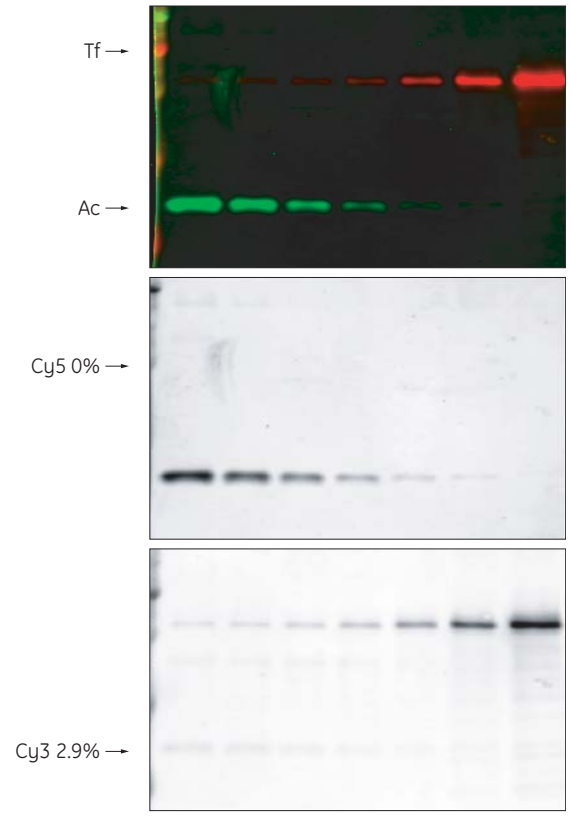


Fig 5. ECL Plex protein detection on Hybond ECL: human apotransferrin (Tf), 5 ng–1.2 pg (four-fold dilutions); bovine cardiac muscle actin (Ac), 150 ng–2.34 ng (two-fold dilutions). Primary antibodies: monoclonal anti-actin and rabbit polyclonal anti–human transferrin; secondary antibodies: ECL Plex goat-α-rabbit IgG-Cy5 and ECL Plex goat-α-mouse IgG-Cy3. False signal in respective channel due to dye interactions is shown (%).

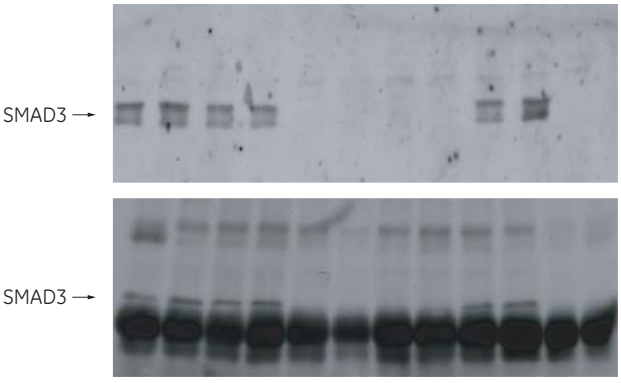


Fig 6. Detection of SMAD3 in cell lysates on Hybond ECL. ECL Plex (top) shows low nonspecific detection compared to the ECL Advance chemi-luminescent kit (bottom). Primary antibody: monoclonal anti-SMAD3; secondary antibody: ECL Plex goat-α-mouse IgG-Cy3.

Ordering information

ECL Plex products

ECL Plex Western blotting combination pack: Cy3™, Cy5, Hybond ECL RPN998

Combination pack optimized for ECL Plex Western blotting, includes Hybond ECL (nitrocellulose membrane). Contains the following components, sufficient for at least 1000 cm² of membrane:

ECL Plex goat-α-mouse IgG-Cy3, 150 µg

ECL Plex goat-α-rabbit IgG-Cy5, 150 µg

ECL Plex Fluorescent Rainbow™ Markers, full-range, 120 µl

Hybond ECL, 10 x 10 cm, 10 sheets

ECL Plex Western blotting combination pack: Cy3, Cy5, Hybond-LFP RPN999

Combination pack optimized for ECL Plex Western blotting, includes Hybond-LFP (low-fluorescent PVDF membrane). Contains the following components, sufficient for at least 1000 cm² of membrane:

ECL Plex goat-α-mouse IgG-Cy3, 150 µg

ECL Plex goat-α-rabbit IgG-Cy5, 150 µg

ECL Plex Fluorescent Rainbow Markers, full-range, 120 µl

Hybond-LFP, 20 x 20 cm, 3 sheets

ECL Plex Cy3- and Cy5-conjugated antibodies

ECL Plex goat-α-mouse IgG-Cy3, 150 µg PA43009
Sufficient for at least 1000 cm² of membrane

ECL Plex goat-α-mouse IgG-Cy3, 600 µg PA43010
Sufficient for at least 4000 cm² of membrane

ECL Plex goat-α-mouse IgG-Cy5, 150 µg PA45009
Sufficient for at least 1000 cm² of membrane

ECL Plex goat-α-mouse IgG-Cy5, 600 µg PA45010
Sufficient for at least 4000 cm² of membrane

ECL Plex goat-α-rabbit IgG-Cy5, 150 µg PA45011
Sufficient for at least 1000 cm² of membrane

ECL Plex goat-α-rabbit IgG-Cy5, 600 µg PA45012
Sufficient for at least 4000 cm² of membrane

Ordering information (continued)

Hybond ECL

Low-fluorescent nitrocellulose membrane, 0.45-µm pore size. Optimized for use with the ECL Plex Western Blotting System.

Hybond ECL, 20 x 20 cm, 10 sheets	RPN2020D
Hybond ECL, 7 x 8 cm, 50 sheets	RPN78D
Hybond ECL, 20 cm x 3 m, 1 roll	RPN203D
Hybond ECL, 30 cm x 3 m, 1 roll	RPN303D
Hybond ECL, 30 cm x 3 m, 1 roll	RPN3032D*

**0.2-µm pore size*

Hybond-LFP

Low-fluorescent PVDF membrane, 0.2-µm pore size. Optimized for use with the ECL Plex Western Blotting System.

Hybond-LFP, 20 x 20 cm, 3 sheets	RPN2020LFP3
Hybond-LFP, 20 x 20 cm, 10 sheets	RPN2020LFP
Hybond-LFP, 14 x 16 cm, 15 sheets	RPN1416LFP
Hybond-LFP, 30 cm x 3 m, 1 roll	RPN303LFP

ECL Plex Fluorescent Rainbow Markers

Full-range, defined molecular weight standards 10–250 kDa. Optimized for use with the ECL Plex Western Blotting System. Supplied in gel loading buffer.

ECL Plex Fluorescent Rainbow Markers, 120 µl	RPN850
ECL Plex Fluorescent Rainbow Markers, 500 µl	RPN851

Blocking buffer

Optimized for use with the ECL Plex Western Blotting System.

BSA, 25 g	RPN412

Related products

ECL Western blotting reagents

ECL Western Blotting System	RPN2108
ECL Plus Western Blotting Detection Reagents	RPN2132
ECL Advance Western Blotting Detection Kit	RPN2135

CyDye Antibody Labeling Kits, using bis-Reactive CyDye

Cy2 Ab Labeling Kit	PA32000
Cy3 Ab Labeling Kit	PA33000
Cy5 Ab Labeling Kit	PA35000

CyDye Value Packs (bis-Reactive NHS esters)

Cy3.5 Bis NHS ester, 5 mg	PA13500
Cy5.5 Bis NHS ester, 5 mg	PA15500
Cy7 Bis NHS ester, 5 mg	PA17000

CyDye Monoclonal Antibody Labeling Kits, using mono-Reactive CyDye

Cy2 mAb Labeling Kit	PA32001
Cy3 mAb Labeling Kit	PA33001
Cy5 mAb Labeling Kit	PA35001

CyDye Value Packs (mono-Reactive NHS esters)

Cy3.5 NHS ester, 1 mg	PA13601
Cy5.5 NHS ester, 1 mg	PA15601
Cy7 NHS ester, 1 mg	PA17101

Gel electrophoresis and transfer equipment

TE 70 PWR ECL Semi-Dry Transfer Unit	11-0013-41
TE 77 PWR ECL Semi-Dry Transfer Unit	11-0013-42
TE 70 ECL Semi-Dry Transfer Unit	80-6210-34
TE 77 ECL Semi-Dry Transfer Unit	80-6211-86
TE62 Transfer Unit	80-6209-58
miniVE Vertical Electrophoresis system	80-6418-77
miniVE blot module	80-6418-96
SE260 Mini-Vertical Unit for two slab gels	80-6149-35
SE600 Ruby Standard Dual Cooled Vertical Unit	80-6479-57
ECL Multiprobe	11-0033-95
ECL Multiprobe XL	11-0033-96
EPS 301 Power Supply	18-1130-01

Scanner and image analysis software

Typhoon 9410 and ImageQuant™ TL	9410-PC

Other

Deep Purple™ Total Protein Stain, 5 ml (makes 1 l)	RPN6305
Deep Purple Total Protein Stain, 25 ml (makes 5 l)	RPN6306

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