

the smart choice for reliable results

Amersham ECL Direct

Fast and convenient system for direct, nonradioactive labeling and detection. Suitable for high- to medium-target applications such as colony/plaque screens, dot blots, and PCR product analysis.



Amersham ECL Direct

ECL Direct™ is the most rapid nonradioactive labeling and detection system available. It is an easy-to-use system for high- to medium-target amounts. ECL Direct employs the convenience of direct enzyme labeling (no blocking or antibody stages) to label DNA or RNA probes with horseradish peroxidase (HRP).

Other features include:

- **Rapid results:** Direct probe labeling in a 10-min reaction, 1 h from hybridization to detection with ECL Direct, Hybond™ N+ and Hyperfilm™ ECL.
- **Ease of handling:** Eliminates handling, waste, and regulatory issues associated with the use of radioactivity.
- **Simple reprobng:** No need to strip blots before reprobng.

This makes ECL Direct the ideal labeling and detection system for applications requiring rapid throughput and fast results.

Using ECL Direct

Probe labeling

To label the probe, denatured single-stranded DNA or RNA probe is added to the labeling solution. The positively charged HRP complex is bound electrostatically to the negatively charged DNA. Glutaraldehyde is added to covalently cross-link the HRP to the probe (Fig 1). The probe can either be used directly in hybridization without purification or it can be stored in 50% glycerol for several months at -15 °C to -30 °C.

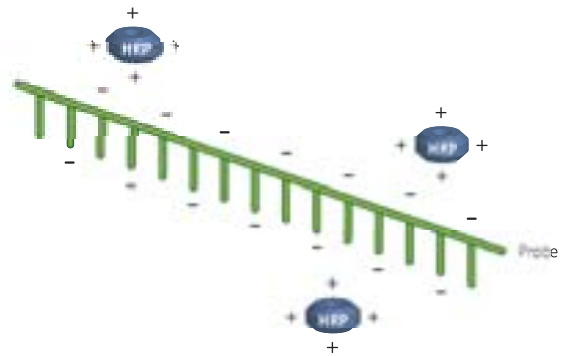


Fig 1. Probe labeling.

Hybridization

Specially formulated ECL Gold Buffer which stabilizes the activity of the enzyme is used for both the 15-min prehybridization and the probe hybridization step, which is typically carried out overnight (Fig 2). For colony or plaque screens, a 2-4 h hybridization is sufficient.

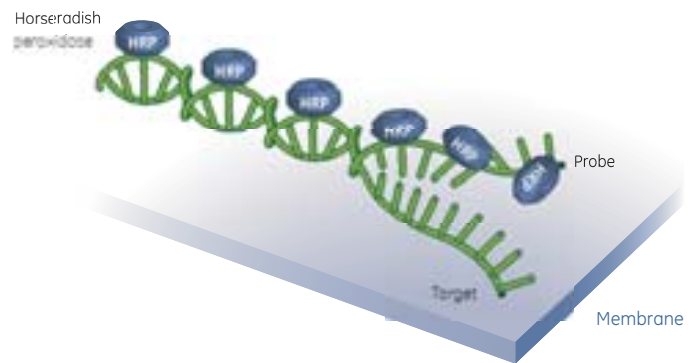


Fig 2. Hybridization.

Detection

For detection, the blots are hybridized for 1 min in the detection reagent which combines luminol—a substrate for HRP—and an enhancer to maximize light output (Fig 3). The blots are then exposed to either Hyperfilm ECL for several minutes to 1 h or a light-capture scanning device. ECL Direct detects down to 500 fg of target DNA.

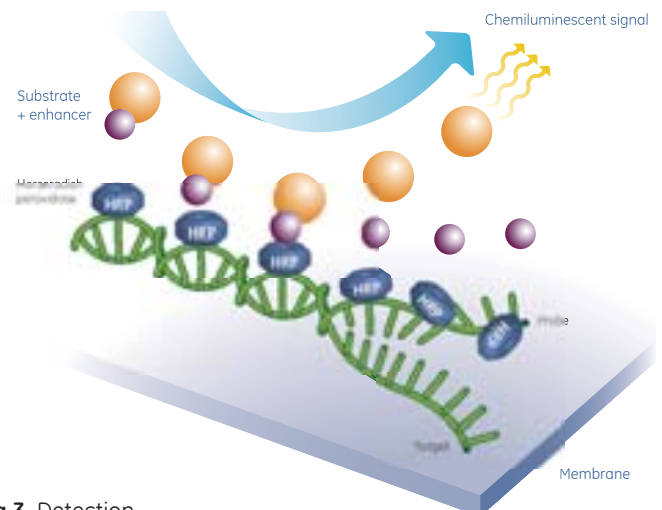


Fig 3. Detection.

Fast and accurate results with Amersham ECL Direct

Strong signals with low background

ECL Direct provides consistent results with the use of ECL Gold Hybridization Buffer. The ECL Gold Hybridization Buffer contains a novel rate enhancer that gives high sensitivity with very low backgrounds on nylon membranes. It also enables lower hybridization temperatures which protect the HRP enzyme.



Fig 4. Random YAC DNA digested with *Pvu* II and hybridized with Alu I human DNA repetitive probe (exposure time of 30 min), using ECL Direct labeling.

Reprobing

HRP is inactivated by the chemiluminescent reaction so there is no need to strip blots before reprobing. This saves time and avoids possible membrane damage, which is often the limiting factor for the number of times a blot can be reprobated.

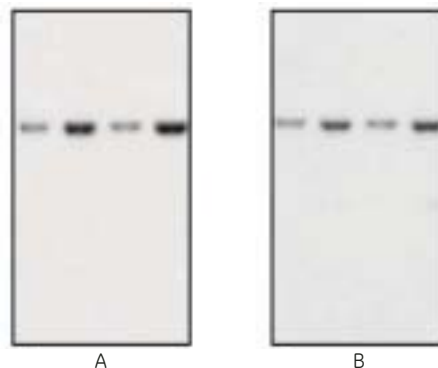


Fig 5. Reprobing. Comparison of 1st and 6th reprobing of Southern-blotted human genomic DNA bound to Hybond-N+. Minimal reduction in signal intensity is observed. (A) 1st reprobing; (B) 6th reprobing.

Recommended applications

ECL Direct is particularly useful for applications that require a blot to be reprobated several times and also for screening applications requiring rapid throughput and fast results. Examples include:

- High- to medium-target Southern and Northern blots
- Colony/plaque lifts
- Dot/slot blots
- Genome mapping
- PCR product analysis
- Library screens

Ordering information

Products	Quantity	Code Number
ECL Direct Labeling and Detection System	To label 5 µg	RPN3000
ECL Direct Labeling and Detection System	To label 10 µg	RPN3001
ECL Direct Labeling module	To label 5 µg	RPN3005
ECL Detection Reagents	For 2000 cm ² membrane	RPN3004
ECL Detection Reagents	For 4000 cm ² membrane	RPN2105
ECL Gold Hybridization Buffer	Sufficient for 4000 cm ² membrane	RPN3006

Related Products

VacuGene™ XL Vacuum Blotting Unit	1	80-1266-24
PR 648 Slot Blot Manifold, 48-well	1	80-6095-58
Hybond-N+	see catalog for full product range	
Hybridization Oven/Shaker 220/240 V	1	RPN2510E
Hybridization Oven/Shaker 110/115 V	1	RPN2511E
Hyperfilm ECL	see catalog for full product range	
Imaging Systems	see catalog for full product range	

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