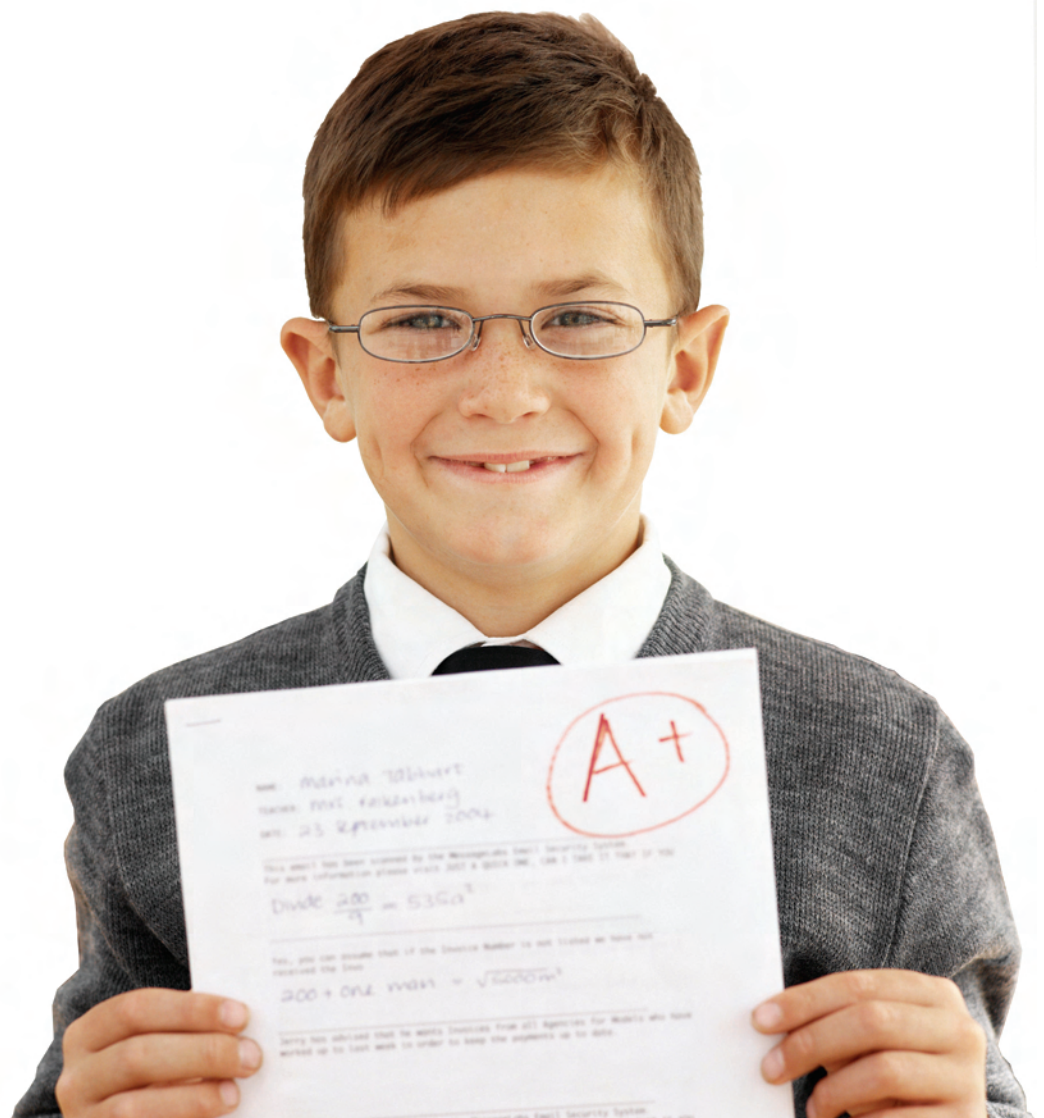


GE Healthcare

the smart choice for reliable results

Amersham AlkPhos Direct

Fast and convenient system for high sensitivity, direct, nonradioactive labeling and detection using DNA, RNA or oligo probes.



Amersham AlkPhos Direct

AlkPhos Direct™ is a fast, sensitive, and easy-to-use system suitable for the majority of routine blotting applications using DNA, RNA or oligonucleotide probes.

Other benefits include:

- **Sensitivity:** AlkPhos Direct with Hybond™ N+ can detect as low as 60 fg target DNA using CDP-*Star*™ reagent (single-copy genomic Southern and Northern blots).
- **Fast results:** 30-min probe labeling, 1 h from hybridization to detection with AlkPhos Direct, Hybond-N+, and Hyperfilm™ ECL.
- **Ease of handling:** Eliminates handling, waste, and regulatory issues associated with the use of radioactivity. Making the correct choice of a nonradioactive system requires consideration of both the labeling and detection procedures.
- **Stringency:** The thermostable nature of the enzyme allows you to control hybridization stringency by varying the temperature as well as salt concentration.
- **Probe stability:** AlkPhos Direct labeled probes are stable for up to six months.

AlkPhos Direct labeling and detection systems are available with either the chemiluminescent detection reagent CDP-*Star* for high sensitivity or the ECF™ detection substrate for fluorescence scanning instrumentation.

Using AlkPhos Direct

Probe labeling

Denatured single-stranded DNA or RNA is mixed with labeling buffer and alkaline phosphatase. Formaldehyde is used to crosslink the enzyme to the probe (Fig 1). The probe can be used directly in hybridization without purification or it can be stored since it is stable for up to 6 months.

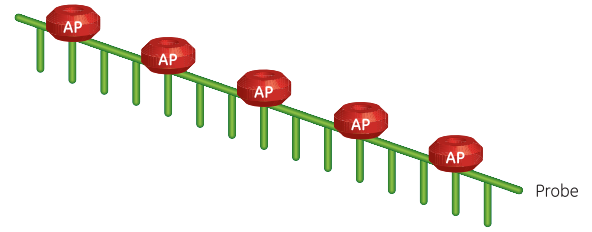


Fig 1. Probe labeling.

Hybridization

Specially formulated AlkPhos Direct hybridization buffer that stabilizes enzyme activity is used for both the 15-min prehybridization and the probe hybridization step, which is typically carried out overnight (Fig 2). For higher target amounts, a 2–4 h hybridization may be sufficient.

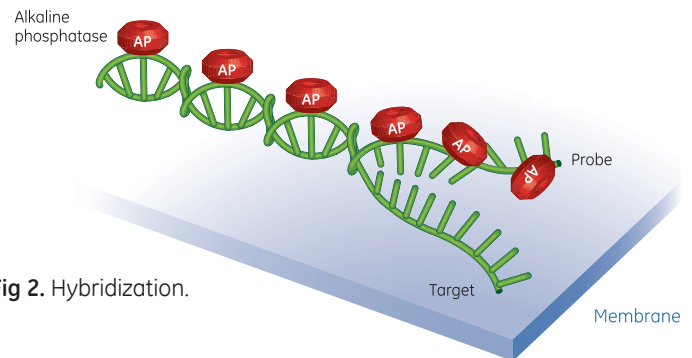


Fig 2. Hybridization.

Detection

AlkPhos Direct can be used with CDP-Star for high sensitivity or with ECF when quantitation is desired. The prolonged light output for up to five days with AlkPhos Direct enables exposure optimization and also allows multiple exposures to be taken.

Chemiluminescent detection with CDP-Star.

For detection, the blots are hybridized for 2 min in the CDP-Star reagent. Blots are then exposed to Hyperfilm ECL for 1–2 h or a light-capture scanning device (Fig 3).

Chemifluorescent detection with ECF.

With this method, a nonfluorescent substrate is catalyzed by alkaline phosphatase to produce a fluorescent signal (Fig 3), which accumulates over time at the site of hybridization. Low-sensitivity applications yield results after 1 h while high-sensitivity applications usually require an overnight incubation. ECF detection is particularly suitable for applications where quantitation is important.

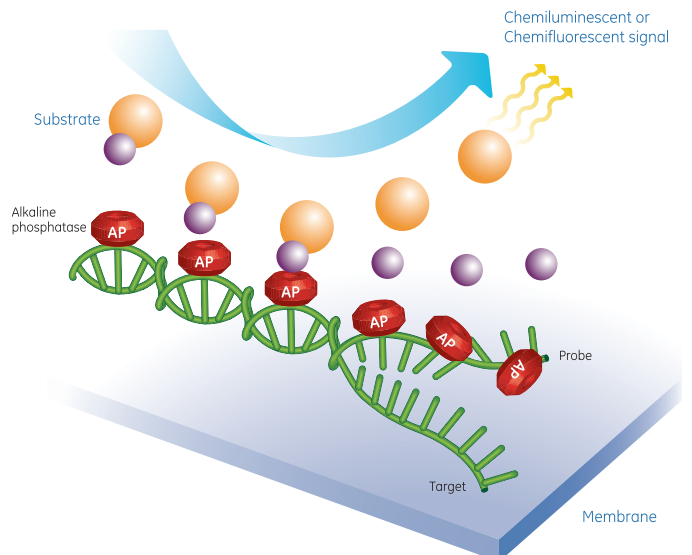


Fig 3. Detection. CDP-Star or ECF detection substrates are incubated with the hybridized blot. Alkaline phosphatase catalyzes the production of either chemiluminescent (CDP-Star) or chemifluorescent (ECF) signals, respectively.

Save time without compromising results

Sensitivity

Results achieved with direct labeling are comparable to those of a hapten-based indirect labeling and detection method (Fig 4). With AlkPhos Direct and the CDP-*Star* reagent, it is possible to detect down to 60 fg of target DNA (single-copy genomic Southern and Northern blots). The accuracy of your experiments is also assured because probe concentrations during hybridization can be determined more precisely. This is because the crosslinking labeling reaction does not result in any net synthesis of the probe.

Time saving

AlkPhos Direct saves 3–4 h over conventional indirect methods by eliminating antibody incubation steps (Fig 5). The system generates fast results within 30 min for probe labeling, and 1 h from hybridization to detection with Hybond-N+ and Hyperfilm ECL.

Stringency control

The thermal stability of AlkPhos Direct enzyme allows dual stringency control using elevated temperature as well as reduced salt concentrations (Fig 6).

Stripping and reprobing

Probe removal is more effective with less damage to the membrane as there are fewer components to be removed during the stripping procedure. Indirect labeling and detection systems require removal of probe and antibody (Fig 7).

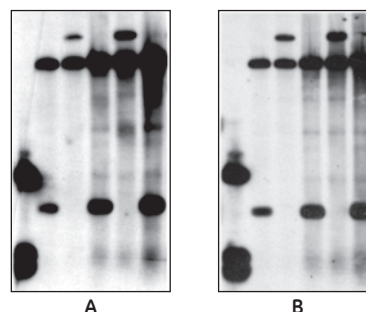


Fig 4. Southern blot. Cosmid DNA digested with *Not* I and *Eco* RI and probed with a 1.1-kb fragment labeled with AlkPhos Direct and a competitor's hapten-based system. (A) AlkPhos Direct (B) Competitor's hapten-based system (Courtesy of Janet Bartels, Yale University).

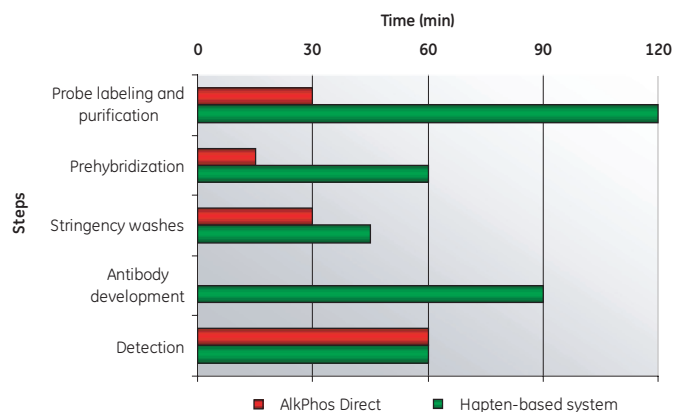


Fig 5. Comparison with a hapten-based system shows that AlkPhos Direct can reduce experimental protocols by up to 4 h.

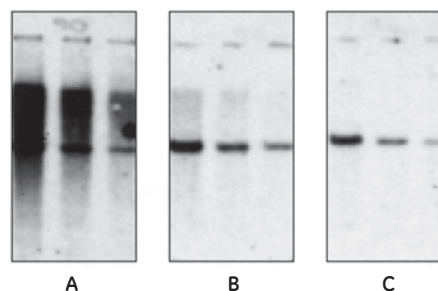


Fig 6. Control of stringency by wash/temperature variations. Human genomic Southern blots (2, 1, and 0.5 μ g loadings) hybridized with BCL2 at 5 ng/ml. Washed at: (A) 50 °C; (B) 55 °C; (C) 60 °C. A 0.2% blocking reagent was included in the primary wash buffer.

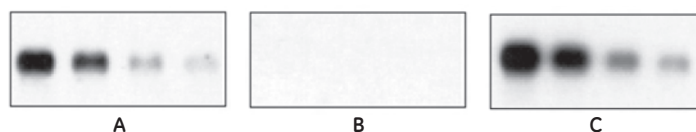


Fig 7. Northern blot (Hybond-N+) of human skeletal muscle (total loadings of 0.5, 0.25, 0.125, 0.063 μ g). Hybridized with *GAPDH* probe. (A) First detection; (B) CDP-*Star* re-applied after stripping; (C) CDP-*Star* re-applied after reprobing.

Recommended applications

AlkPhos Direct is a fast and easy-to-use system for high-sensitivity applications using either DNA or RNA probes. Examples include:

- Single-copy genomic Southern blots
- Northern blots
- Labeling of oligonucleotide probes
- Applications requiring quantitation, for example on a fluorescence scanner such as Typhoon™ 9410
- High-throughput, membrane-based screening applications

Ordering information

Products	Quantity	Code Number
AlkPhos Direct Labeling and Detection System with CDP- <i>Star</i>	For 2500 cm ² membrane	RPN3690
AlkPhos Direct Labeling and Detection System with CDP- <i>Star</i>	For 5000 cm ² membrane	RPN3691
AlkPhos Direct Labeling and Detection System with ECF	For 2500 cm ² membrane	RPN3692
AlkPhos Direct Labeling module	For 2500 cm ² membrane	RPN3680
AlkPhos Direct Labeling module	For 5000 cm ² membrane	RPN3681
CDP- <i>Star</i> Detection Reagent	For 2500 cm ² membrane	RPN3682
CDP- <i>Star</i> Detection Reagent	For 5000 cm ² membrane	RPN3683
ECF Detection module	For 2500 cm ² membrane	RPN3685
AlkPhos Direct Hybridization Buffer	For 5000 cm ² membrane	RPN3688

Related Products

Hybond-N+	see catalog for full product range
Hyperfilm ECL	see catalog for full product range
Typhoon 9410 with PC	63-0055-81

General Electric Company reserves the right, subject to any regulatory approval if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation. Contact your GE Representative for the most current information.

AlkPhos Direct, Amersham, ECF, Hybond, Hyperfilm, and Typhoon are trademarks of GE Healthcare Companies.

CDP-Star is a trademark of Tropix Inc.

© 2006 General Electric Company—All rights reserved. GE and GE Monogram are trademarks of General Electric Company.

Asia Pacific

T +85 65 62751830
F +85 65 62751829

Australasia

T +61 2 8820 8299
F +61 2 8820 8200

Austria

T 01 /57606 1613
F 01 /57606 1614

Belgium

T 0800 73 890
F 02 416 8206

Canada

T 1 800 463 5800
F 1 800 567 1008

Central & East Europe

T +43 1 972 720
F +43 1 972 722 750

Denmark

T +45 70 25 24 50
F +45 45 16 2424

Eire

T 1 800 709992
F +44 1494 542010

Finland & Baltics

T +358 9 512 3940
F +358 9 512 39439

France

T 01 69 35 67 00
F 01 69 41 98 77

Germany

T 0800 9080 711
F 0800 9080 712

Greater China

T +852 2100 6300
F +852 2100 6338

Italy

T 02 26001 320
F 02 26001 399

Japan

T 81 3 5331 9336
F 81 3 5331 9370

Korea

T 82 2 6201 3700
F 82 2 6201 3803

Latin America

T +55 11 3933 7300
F +55 11 3933 7304

Middle East & Africa

T +30 210 96 00 687
F +30 210 96 00 693

Netherlands

T 0800-82 82 82 1
F 0800-82 82 82 4

Norway

T +47 815 65 777
F +47 815 65 666

Portugal

T 21 417 7035
F 21 417 3184

Russia, CIS & NIS

T +7 495 956 5177
F +7 495 956 5176

Spain

T 902 11 72 65
F 935 94 49 65

Sweden

T 018 612 1900
F 018 612 1910

Switzerland

T 0848 8028 10
F 0848 8028 11

UK

T 0800 515 313
F 0800 616 927

USA

T +1 800 526 3593
F +1 877 295 8102

www.gehealthcare.com/na_blotting

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK



imagination at work