The Guide to Nucleic Acid Labelling and Detection

Amersham Biosciences

Contents

Introduction

For many years the use of radiolabels was the method of choice for nucleic acid labelling and detection. More recently, however, considerable research has lead to the development of a comprehensive range of quality systems using both radioactive and non-radioactive technologies. This extensive range now provides you with an excellent choice of systems for use with DNA, RNA and oligonucleotides in a variety of applications.

Significant developments in the area of non-radioactive labelling and detection include the use of light-based detection systems and direct labelling. The introduction of these techniques means that non-radioactive methods now present a viable alternative to the use of traditional radioactive techniques for many applications.

This breadth of choice can in itself cause problems. Choosing the most appropriate system for a particular application can be a challenge for even the most experienced researchers.

The choice is influenced by many factors, not all of which are directly related to the application. For example radioactive labelling has the advantage of high sensitivity and extremely robust protocols, however safety and long-term probe storage are serious considerations.

The aim of this guide is to give an overall introduction to the different radioactive and non-radioactive methodologies available, highlighting their advantages and drawbacks. Individual products are then presented in more detail allowing you to choose the most appropriate system for your application.

Factor	**Considerations**
Blotting system	Southern, Northern, dot/slot, library screen. Amount of target nucleic acid present
Label	Radioactive or non-radioactive Direct or indirect
Probe	DNA, RNA, oligonucleotide Size and purity of probe
Speed and convenience	Multiple exposures Time constraints
Probe storage	Stability
Results	Autoradiography film Image analysis with scanners
Membrane	Charged or uncharged nylon Nitrocellulose

Table 1 Factors affecting the choice of labelling and detection system

Choosing a non-radioactive labelling system

In the past the use of non-radioactive labelling and detection technologies has been limited by the relatively low levels of detection sensitivity as compared with radioactive detection. However, the further development of non-radioactive labelling methods, coupled with light-based detection has resulted in systems that now offer a real alternative to the use of radioisotopes in many applications where target levels are 50 fg and above.

Non-radioactive systems offer the following benefits:

Speed	Exposure times with non-radioactive systems range from minutes to hours.
Sensitivity	Applications where target levels are 50 fg and above, e.g. single copy Southern and Northern blotting, colony/plaque blots.
Stable probes	Non-radioactively labelled probes are stable for at least 6 months, removing the need to label probes immediately before use.
Ease of handling	Eliminates handling and waste regulatory issues associated with the use of radioactivity.

Making the correct choice of non-radioactive system requires consideration of both the labelling and detection procedures.

Labelling

Non-radioactive labelling methods are separated into two different approaches. Early non-radioactive systems utilized indirect labelling techniques, but these have now been largely superseded by the introduction of superior direct labelling methods, an example of which is AlkPhos Direct™.

Direct labelling Direct labelling* using alkaline phosphatase is the most recent advance in the non-radioactive labelling of nucleic acids. It offers significant improvements in speed and convenience over indirect systems without compromising sensitivity.

With direct labelling systems, the enzyme molecule (alkaline phosphatase or horseradish peroxidase) is directly crosslinked to the nucleic acid probe in a simple 30-minute reaction.

Detection is rapid because direct labelling methods eliminate the need for an antibody conjugate incubation and the associated blocking and multiple washing steps.

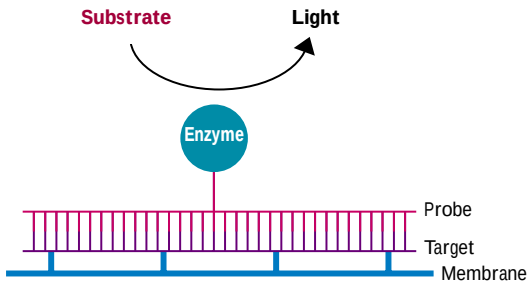

Figure 1 Direct labelling

*For licence information see page 37

Choosing a non-radioactive labelling system

Indirect labelling Indirect labelling involves the introduction into the probe of nucleotides tagged with a hapten or 'reporter' molecule. Amersham Biosciences kits use fluorescein as the hapten molecule. The nucleotide analogues are readily incorporated into the probe by standard labelling methods and hybridization and stringency washing are carried out under standard conditions. Labelled probes are then detected with a highly specific anti-fluorescein antibody conjugated to either alkaline phosphatase (AP) or horseradish peroxidase (HRP) enzyme. This is followed by enzyme catalysed detection, using the appropriate substrate.

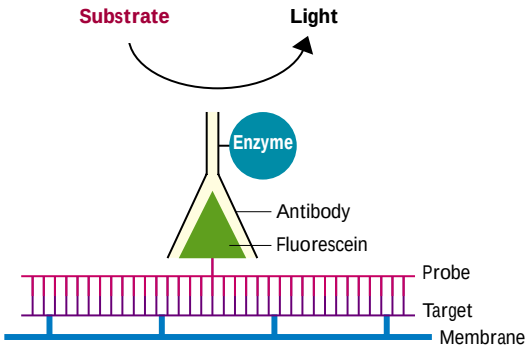

Figure 2 Indirect labelling

Detection

Choice of detection method will depend on the enzyme used in the labelling stage as well as the detection medium required. Horseradish peroxidase can only be detected using a chemiluminescent substrate, but alkaline phosphatase can be detected using chemiluminescent and chemifluorescent substrates. Chemiluminescent detection systems are designed for use with autoradiography film whereas fluorescence based detection systems require the use of suitable scanning equipment.

ECL

Based on the enhanced chemiluminescent reaction of luminol with horseradish peroxidase, ECL™ substrate can be used to detect probes which have been labelled either directly or indirectly with horseradish peroxidase. Rapid light output enables results to be achieved in 10 to 15 minutes. It is the substrate of choice for target amounts above 500 fg.

CDP-*Star*

Based on the chemiluminescent breakdown of the dioxetane CDP-*Star*™ by alkaline phosphatase*, this substrate can be used to detect probes which have been labelled either directly or indirectly with alkaline phosphatase. It is ideal for applications requiring high sensitivity detection such as genomic Southern and Northern blots.

Light output is rapid and continues for up to five days allowing exposure optimization and multiple exposures to be taken.

ECF

Based on the breakdown of the ECF™ substrate by alkaline phosphatase*, resulting in a highly fluorescent product which is localized at the site of hybridization. This chemifluorescent signal is detected using a suitable fluorescence scanner such as the Amersham Biosciences™ FluorImager™ or Storm™. ECF detection can be used to detect probes which have been either directly or indirectly labelled with alkaline phosphatase and is particularly suitable for applications where quantification is important.

Labelling and detection system	Sensitivity	Time from hybridization to detection	Duration of light output	Strip before re-probing	Quantification	Recommended application
AlkPhos Direct	0.06 pg	1 hour	5 days	yes	no	Single copy Southern and Northern
ECL Direct	0.5 pg	1 hour	1-2 hours	no	no	High target applications e.g. colony/plaques
Gene Images Random-Prime	0.1 pg	3 hours	5 days	yes	no	High sensitivity Northerns
Gene Images 3'- end labelling with CDP-*Star*	0.1 pg	3 hours	5 days	yes	no	Oligo screening with stringency control
Gene Images 3'- end labelling with ECF	120 pg	3 hours	1-2 days	yes	yes	Quantification
ECL Random-Prime	0.5 pg	3 hours	1-2 hours	yes	no	Medium target Southern with DNA probes
ECL 3'-end labelling	0.2 pg	3 hours	1-2 hours	yes	no	Medium to high target Southern with oligo probes
ECF Random-Prime	0.25 pg	3 hours	1-2 days	yes	yes	Quantification

Table 2 Guide to the properties of non-radioactive labelling and detection systems

*For licence information see page 37.

AlkPhos Direct Labelling and Detection

AlkPhos Direct™ combines the convenience of direct enzyme labelling (no blocking or antibody stages) with those of alkaline phosphatase detection (long light output and high sensitivity).

Labelling is complete within 30 minutes in a single tube protocol and the resulting probe is ready for use in hybridizations without further purification.

Due to the thermostable nature of the enzyme, hybridization stringency can be controlled by adjusting temperature as well as salt concentration.

The system can be used with either chemiluminescent or fluorescent detection reagents. The most sensitive result is achieved with CDP-*Star*, a chemiluminescent substrate with a fast light output. Alternatively, the use of ECF substrate generates a fluorescent signal suitable for use with fluorescence scanning devices such as Amersham Biosciences FluorImager or Storm instruments.

Protocol Summary

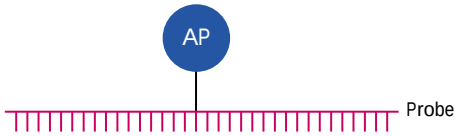

Figure 3 Probe labelling

Probe labelling

Denatured or single-stranded DNA or RNA probe is mixed with the labelling buffer and alkaline phosphatase. Formaldehyde is then added to covalently crosslink the enzyme to the probe. There is no need to purify the probe before hybridization.

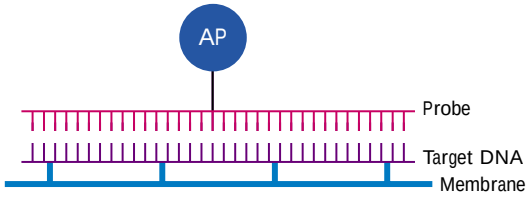

Figure 4 Hybridization

Hybridization and stringency

The probe is hybridized to the blot using the specially formulated AlkPhos Direct hybridization buffer, which stabilizes the activity of the enzyme. After a 15 minute pre-hybridization step, hybridization with probe is performed overnight at the required temperature. For higher target amounts, a 2 to 4 hour hybridization may be sufficient.

Systems

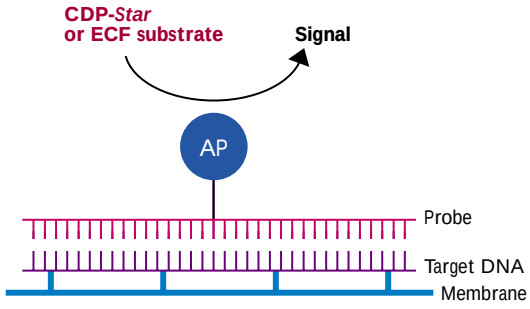

Figure 5 Detection

Figure 5 Detection

Detection

Chemiluminescence with CDP-*Star*:

Hybridized blots are detected with CDP-*Star* reagent. Following a two-minute incubation, blots are exposed to Hyperfilm™ MP for 1 to 2 hours or a light capture scanning device. Sensitivities as low as 60fg can be achieved using CDP-*Star*.

or

Chemifluorescence with ECF:

Hybridized blots are detected with ECF substrate. Non-fluorescent substrate is catalysed by alkaline phosphatase to produce a fluorescent signal which accumulates over time; low sensitivity applications yield results after 1 hour, high sensitivity applications usually require overnight incubation.

Recommended Applications

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

The versatility of the system, combined with the fact that both DNA and RNA probes are labelled equally efficiently, makes it the ideal choice for a busy laboratory carrying out a variety of different blotting and hybridization techniques.

For Southern and Northern blotting applications AlkPhos Direct, combined with CDP-*Star* detection, offers a highly sensitive system capable of detecting down to 60 fg of target DNA. Thus making it the ideal choice for the non-radioactive detection of single-copy genomic Southern blots.

Re-probing can be difficult with non-radioactive systems due to the need to remove both probe and antibody layers in an indirect system. The simplicity of the AlkPhos Direct system means that there are less components to be removed during the stripping procedure. Probe removal is therefore more effective with less damage to the membrane.

For labelling oligonucleotides, an optimized protocol has been developed which is capable of labelling probes down to 17 basepairs in length. Request TechTip number 174 for additional information.

Quantification of dot and slot blots is possible by using ECF detection in conjunction with AlkPhos Direct. Analysing results on a fluorescence scanner such as Amersham Biosciences Storm or FluorImager allows accurate quantification and analysis of results.

AlkPhos Direct Labelling and Detection

Speed Time savings of 3 to 4 hours over conventional indirect methods are achievable as a result of the rapid and simple labelling reaction, and the absence of antibody incubations.

Consistency Quality control on each batch ensures consistent performance and provides confidence in probe labelling and concentration.

Stringency control Due to the thermostable nature of the enzyme, hybridization stringency can be controlled by elevating temperatures up to 75°C as well as by decreasing salt concentrations.

Versatility Labelling of DNA, RNA and oligonucleotide probes with detection by chemiluminescence or chemifluorescence offers a high degree of versatility.

Stability Probes are stable for up to six months if stored at –20°C in 50% glycerol.

Efficiency Nucleic acid probes are labelled efficiently and uniformly with enzyme.

Accuracy The crosslinking labelling reaction does not result in any net synthesis of probe and the amount of probe present before and after labelling does not change. Therefore probe concentrations during hybridization can be determined more accurately.

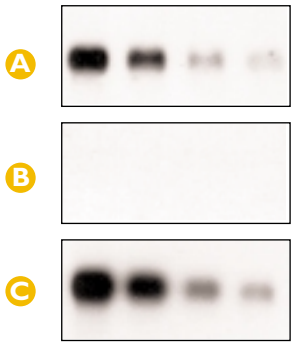

Figure 7
Northern blot (Hybond-N+) of human skeletal muscle (total loadings of 0.5, 0.25, 0.125, 0.063 µg). Hybridized with GAPDH probe.
(A) First detection
(B) CDP-Star re-applied after stripping
(C) CDP-Star re-applied after re-probing

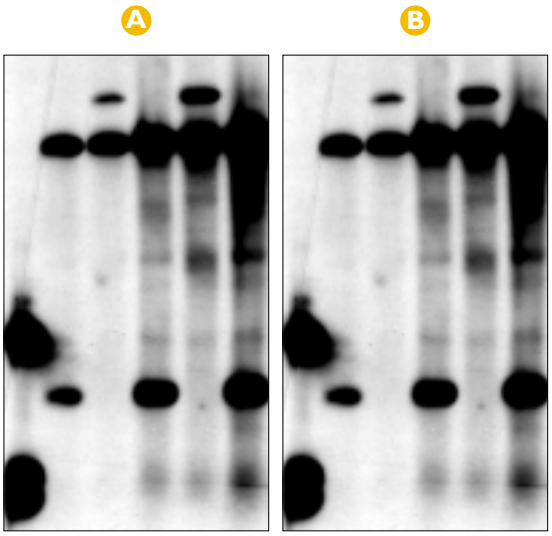

Figure 6
Southern blot
Cosmid DNA digested with Not I and EcoR I, probed with a 1.1kb probe labelled with AlkPhos Direct and a competitor's hapten-based system
(A) AlkPhos Direct
(B) Competitor's hapten-based system
Courtesy of Janet Bartels, Yale University

Systems

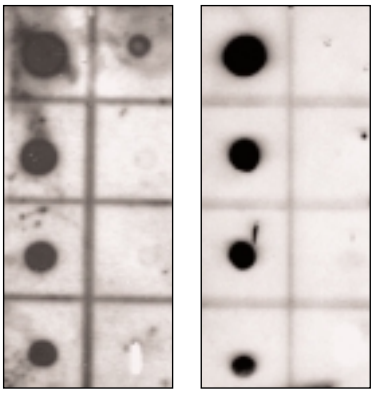

Figure 8

Dot blots loaded with 5, 2, 1, 0.5, 0.1 and 0.01ng of a 1.5kb N-ras insert on Hybond-N+ nylon membrane. The 7th dot was a control of 5ng carrier DNA. Results were obtained with CDP-Star detection reagent.

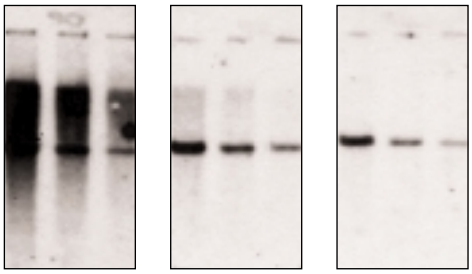

Figure 9

Control of stringency by wash temperature. Human genomic Southern blots (2, 1 and 0.5µg loadings) hybridized with BCL2 at 5ng/ml. Washed at:

(A) 50°C (B) 55°C (C) 60°C

0.2% blocking reagent included in the primary wash buffer.

Ordering Information

AlkPhos Direct Labelling and Detection System with CDP-*Star*	25 reactions	RPN 3690
AlkPhos Direct Labelling and Detection System with CDP-*Star*	50 reactions	RPN 3691
AlkPhos Direct Labelling and Detection System with ECF	25 reactions	RPN 3692
AlkPhos Direct Labelling Module	25 reactions	RPN 3680
AlkPhos Direct Labelling Module	50 reactions	RPN 3681
AlkPhos Direct Hybridization Buffer	For 5000 cm^2 membrane	RPN 3688
CDP-*Star* Detection Reagent	For 2500cm^2 membrane	RPN 3682
CDP-*Star* Detection Reagent	For 5000cm^2 membrane	RPN 3683
ECF Substrate	For 2500cm^2 membrane	RPN 3685

Related products

ECL Direct Nucleic Acid Labelling and Detection System	RPN3000
Hybond-N+ Membranes	see page 28
Hyperfilm MP	see page 30

Gene Images Random-Prime Labelling and Detection

Gene Images™ Random-Prime is designed to generate fluorescein labelled DNA probes using a random prime labelling reaction. The reaction requires only 50 ng of template DNA as the strand displacement properties of the enzyme, coupled with non-limiting concentrations of nucleotide, result in net synthesis of probe. The use of fluorescein allows the efficiency of the reaction to be checked before hybridization by a fluorescence assay.

Protocol Summary

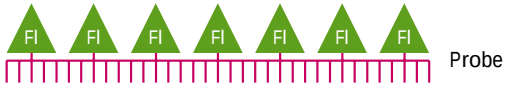

Figure 10 Probe labelling

Probe labelling

Fluorescein dUTP is incorporated into DNA in a random prime labelling reaction using Klenow DNA polymerase. Nonamer primers are used to ensure efficient hybridization to probe. Probes can be used without purification.

Optional rapid labelling assay

The efficiency of probe labelling can be checked by spotting a small aliquot of labelled probe onto Hybond™-N+ membrane, washing to remove unincorporated fluorescein and visualizing on a UV transilluminator or fluorescent scanner.

Hybridization

For optimum performance use the recommended hybridization buffer.

Sensitive detection is achieved by the use of a monoclonal antibody which is highly specific for alkaline phosphatase. 50 fg of target can be routinely detected in a human genomic Southern blot.

Detection

Chemiluminescence with CDP-*Star*:
Hybridized blots are detected with CDP-*Star* reagent. Following a 2 minute incubation, blots are exposed to Hyperfilm MP for 1 to 2 hours. Sensitivities as low as 50 fg can be detected.

or

Chemifluorescence with ECF:
Hybridized blots are detected with ECF substrate. Non-fluorescent substrate is catalysed by alkaline phosphatase to produce a fluorescent signal which accumulates over time; low sensitivity applications yield results after 1 hour, high sensitivity applications usually require overnight incubation.

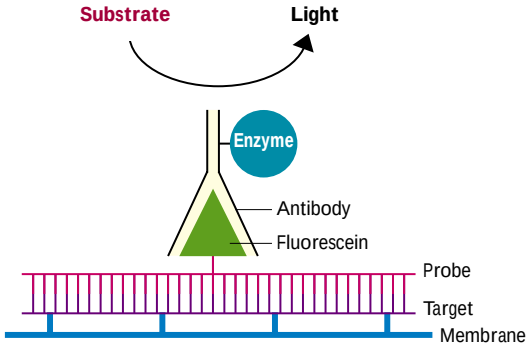

Figure 11 Indirect labelling

Recommended Applications

Probes labelled with the Gene Images Random-Prime Labelling and Detection System can be used for most membrane applications especially in situations where there is insufficient probe for labelling by direct methods.

Gene Images Random-Prime Labelling and Detection Systems may be used with either the detection reagent CDP-*Star* for high sensitivity applications, or with ECF substrate for quantification on fluorescence scanning instrumentation.

Gene Images Random-Prime is especially recommended for high sensitivity Northern applications.

Systems

Confidence The use of fluorescein-dUTP allows a simple and rapid (30 minute) probe labelling assay to be performed by monitoring the fluorescence of labelled probes on a UV transilluminator. This enables the efficiency of the labelling reaction to be checked before proceeding with the hybridization.

Stability Probes are stable for at least 9 months under recommended conditions minimizing the requirement for continual labelling of frequently used probes.

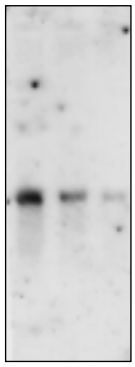

Figure 12
5, 2, 1 and 0.5 µg of human liver mRNA were blotted onto Hybond-N+, fixed using the Amersham crosslinker and hybridized with a fluorescein-labelled DNA proge, specific for p53, at 65°C. CDP-Star detection, 1 hour exposure on Hyperfilm-MP.

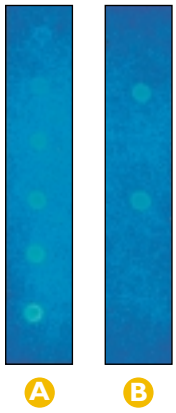

Figure 13
Rapid labelling assay
(a) Serial dilutions of fluorescein nucleotide 1/25, 1/50, 1/100, 1/250, 1/500, 1/1000. Membrane not washed
(b) Aliquots of labelling reactions:
(i) labelling reaction inhibited with EDTA at time 0
(ii) labelled lambda DNA digested with Hind III
(iii) labelled N-ras clone
Membrane washed in 2xSSC at 60°C for 15 minutes. Visualized on a UV transilluminator

Ordering Information

Gene Images Random-Prime Labelling and Detection System	30 reactions	RPN 3500
Gene Images Random-Prime Labelling and Detection System	60 reactions	RPN 3501
Gene Images Random-Prime Labelling Module	30 reactions	RPN 3540
Gene Images Random-Prime Labelling Module	60 reactions	RPN 3541
CDP-*Star* Detection Module	For 2500cm^2 membrane	RPN 3510
CDP-*Star* Detection Module	For 5000cm^2 membrane	RPN 3511
ECF Dectection Module	For 2500cm^2 membrane	RPN 5750

Related products

AlkPhos Direct Labelling and Detection System	RPN3690
Hybond-N+ membranes	see page 28

Gene Images 3'-Oligolabelling and Detection

Gene Images™ 3'-Oligolabelling system is based on the labelling of standard unmodified oligonucleotide sequences with fluorescein-dUTP in a reaction catalysed by terminal deoxynucleotidyl transferase. The optimized protocol results in a tail of 6-8 nucleotides, which provides good sensitivity with low background, and does not interfere with the specificity of hybridization. The use of fluorescein as the label allows the efficiency of the labelling reaction to be checked using a fluorescence assay before hybridization.

Protocol Summary

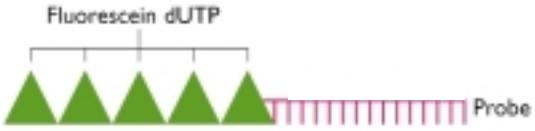

Figure 14 Probe labelling

Probe labelling

Standard oligonucleotides are tailed with fluorescein-dUTP using the enzyme terminal deoxynucleotidyl transferase. There is no need to purify the probe before hybridization.

Optional rapid labelling assay

Labelling efficiency can be checked by spotting a small aliquot of labelled probe onto Hybond-N+ membrane, washing to remove unincorporated fluorescein and visualizing on a transilluminator or fluorescence scanner.

Hybridization

For optimum performance use the recommended hybridization buffer.

Detection

Chemiluminescence with CDP-*Star*:

Hybridized blots are detected with CDP-*Star* reagent. Following a two minute incubation, blots are exposed to autoradiography film for 1 to 2 hours or detected using a light collection device.

or

Chemifluorescence with ECF:

Hybridized blots are detected with ECF substrate. Non-fluorescent substrate is catalysed by alkaline phosphatase to produce a fluorescent signal which accumulates over time; low sensitivity applications yield results after 1 hour; high sensitivity applications usually require overnight incubation.

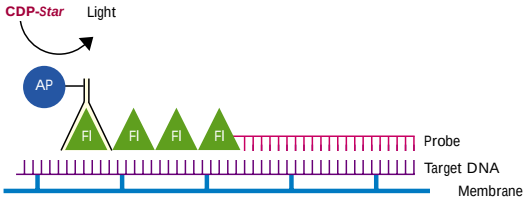

Figure 15 Gene Images 3'-Oligolabelling with CDP-Star detection

System

Recommended Applications

The 3'-Oligolabelling system can be used to label probes for a wide variety of applications. The indirect labelling method and high sensitivity makes it especially suited to oligo screening applications requiring a high degree of stringency control.

Ease of use Simple protocols, and ready-to-use reagents ensures first-time success.

Confidence The use of fluorescein-dUTP allows a simple and rapid (30 minute) probe labelling assay to be performed by monitoring the fluorescence of labelled probes on a UV transilluminator. This enables the efficiency of the labelling reaction to be checked before proceeding with the hybridization. Use with any oligonucleotide, special synthesis is not required.

Stability Labelled probes are stable for at least 9 months, under recommended conditions allowing batches of probe to be prepared and stored until needed.

Figure 16
Dot blots of M13 mp8 (500 - 0.625 attomoles). 3'-end labelled M13 17mer oligonucleotide probe. 5 ng/ml, 2 hour hybridization and detected using CDP-Star. 1 hour exposure to Hyperfilm MP.

Ordering Information

Gene Images 3'-Oligolabelling and CDP-*Star* Detection System	To label 1000 pmol oligo	RPN5776
Gene Images 3'-Oligolabelling Module	To label 1000 pmol oligo	RPN5770
CDP-*Star* Detection Module	For 2500 cm^2 membrane	RPN3510
CDP-*Star* Detection Module	For 5000 cm^2 membrane	RPN3511
ECF Detection Module	For 2500 cm^2 membrane	RPN5750

Related products

AlkPhos Direct Labelling and Detection System with CDP-*Star*	RPN3690

ECL Direct Labelling and Detection System

The ECL Direct™ nucleic acid labelling and detection system is based on the direct labelling of DNA or RNA probes with the enzyme horseradish peroxidase (HRP) in a simple chemical reaction. Labelling takes only 10 minutes in a single tube reaction, and the resulting probe can be used directly in hybridization experiments without further purification. Detection of hybridized probe is achieved by generation of light via HRP catalysed oxidation of luminol with the use of an enhancer maximizing light output.

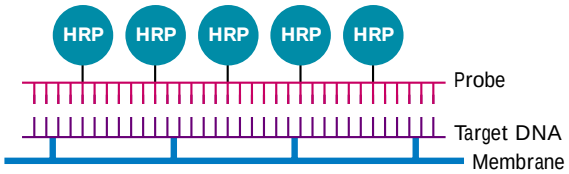

Figure 18

Hybridization

The labelled probe is hybridized to the blot using the specially formulated ECL Gold Buffer, which stabilizes the activity of the enzyme. Following a 15 minute pre-hybridization, hybridization with probe is performed overnight. For colony or plaque screens a 2 to 4 hour hybridization is sufficient.

Protocol Summary

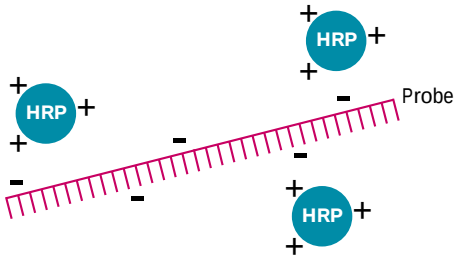

Figure 17 Probe labelling

Probe labelling

The labelling solution is added to the unlabelled, denatured single-stranded DNA or RNA probe. 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. There is no need to purify the probe before hybridization. Labelled probes can be stores in 50% glycerol for several months at -15°C to -30°C.

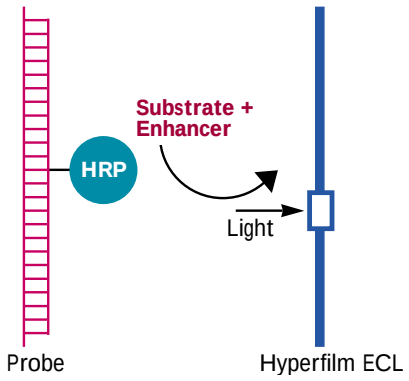

Figure 19 Detection

Detection

The hybridized blot is soaked in the detection reagent. Where the probe is bound there is peroxidase-catalysed oxidation of luminol and subsequent enhanced chemiluminescence. The resulting light signal is detected on autoradiography film.

Recommended Applications

ECL Direct™ is a fast and easy system for high to medium target amount applications such as colony/plaque screens, dot blots, genome mapping, and PCR* product analysis. It is particularly useful for applications which require a blot to be re-probed several times and screening applications requiring rapid throughput and results.

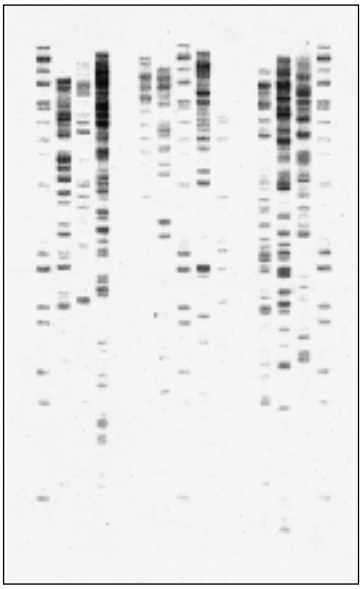

Figure 20
Random YAC DNA digested with PvuII and hybridized with AluI human DNA repetitive probe (exposure time of 30 minutes), using ECL Direct labelling.

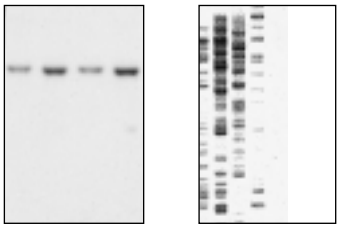

Figure 21
Comparison of 1st and 6th re-probing of Southern blotted human genomic DNA bound to Hybond-N+. Minimal reduction in signal intensity is observed.
A) 6th re-probing
B) 1st probing

Ease of use Simple protocol coupled with high initial signal output makes it the most rapid non-radioactive system available.

Speed Less than 4 hours from probe labelling to detection in high target amount applications with no lengthy antibody steps.

Ease of re-probing HRP is inactivated by the chemiluminescent reaction therefore no need to strip blots before reprobing. This saves time and avoids possible membrane damage.

Consistency Consistent results combining strong signals with very low backgrounds on nylon membranes.

Convenience Convenient, ready-to-use ECL Direct Gold hybridization buffer formulated with an exclusive rate enhancer*.

Efficiency DNA or RNA probes are labelled equally efficiently.

Stability Probes can be stored for at least 3 months in 50% glycerol, avoiding the need for frequent labelling.

Ordering Information

ECL Direct Labelling and Detection System	To label 5 µg nucleic acid	RPN 3000
ECL Direct Labelling and Detection System	To label 10 µg nucleic acid	RPN 3001
ECL Direct Detection Reagents	For 2000cm² membrane For 4000cm² membrane	RPN 3004 RPN 2105
ECL Direct Gold Buffer	For 4000cm² membrane	RPN 3006
ECL Direct Labelling System	To label 5 ng nucleic acid	RPN 3005

Related products

AlkPhos Direct Labelling and Detection System	RPN3690

*For licence information see page 37

Choosing a radioactive labelling system

Labelling with ^{32}P-labelled nucleotides remains the most sensitive and robust technology available. Radiolabelled probes offer the following benefits:

Sensitivity ^{32}P-labelled probes offer the highest level of sensitivity, ideal for detection where there is a very low abundance of target.

Prolonged emission Radioactively labelled blots continue to emit signal for several weeks enabling multiple exposures and long exposure times to be carried out as required.

Reliability Radioactive labelling systems are very forgiving. Precise optimization of hybridization conditions is less critical, and hence less time is used determining ideal hybridization and stringency conditions.

Flexibility Incorporation of radiolabelled nucleotides into a probe can be controlled by the labelling conditions. Therefore probes can be produced with differing specific activities and incorporation levels can be measured accurately, allowing exact probe concentrations to be determined.

Figure 22
Ready-To-Go pre-dispensed reagent beads

Choice of radiolabel

The selection of radiolabel for a particular application depends on the level of sensitivity and resolution required. For most membrane hybridization techniques sensitivity is most important, so a high-emission energy label will provide sensitive results with shorter exposure times but resolution will be compromised.

In applications where resolution is most important, such as microsatellite analysis and sequencing, a lower energy-emitting isotope will give improved resolution, but longer exposure times will be required.

The three most widely used isotopes in the life science field are:

^{32}P For high-sensitivity membrane applications ^{32}P is the label of choice due to its high β-energy and the elevated specific activity of ^{32}P-labelled probes. Labelled nucleotides with a specific activity of >110 TBq/mmol, >3000 Ci/mMol are available for these applications.

^{33}P With the advantage of a lower emission energy, ^{33}P offers increased resolution as compared to ^{32}P. It may be used for membrane hybridizations, but its lower energy emission means that longer exposure times are required. It is ideally suited to sequencing and *in situ* hybridization applications.

^{35}S This label can be used for hybridizations but is not recommended because of its low emission energy. However, it provides high resolution and is suited to sequencing and *in situ* hybridization applications.

Choice of labelling method

Random prime The most widely used method for the uniform labelling of DNA to provide high label density probes.

Ready-To-Go™ DNA Labelling Beads, Rediprime™ II, Megaprime™ and Multiprime™ are all based on the random prime technique which uses random sequence hexamers or nonamers to prime DNA synthesis on a denatured DNA template at numerous sites along its length.

The reaction uses the Klenow fragment of DNA polymerase I and leads to an efficient use of labelled nucleotides and net synthesis of probe. Therefore, very small amounts of input DNA are required, enabling high specific activity DNA probes to be produced with relatively small quantities of added label.

Nick translation This method provides uniformly labelled DNA probes by the introduction of random nicks into the template by the action of DNase I and subsequent filling in by DNA polymerase I. The existing nucleotide sequence of the DNA probe is renewed without net synthesis occurring. Nick translation can be used for the production of large amounts of high-specific activity probes.

End labelling This is the method of choice for labelling oligonucleotide probes. Either the 3' or 5' end can be labelled by the use of different techniques. End-labelled oligonucleotide probes may be used for membrane hybridizations. Probes labelled with ^{35}S and ^{33}P are suitable for use with *in situ* hybridization.

RNA labelling This technique produces radiolabelled RNA probes from inserts cloned into appropriate vectors. SP6 and T7 RNA polymerase may be used to produce asymmetric probes from the same or different vectors. Asymmetric probes are used in *in situ* hybridization applications to provide both positive results and negative controls.

Figure 23 Redivial™ showing removal of the splash guard which secures the radioactive solution at the bottom of the vial during ambient shipment. The splash guard is easily removed and disposed of as active waste.

Labelling system	Technology	Nucleotide	Amount of template	Labelling time	Probe specific activity (dpm/μg)	Recommended application
Rediprime II	Random-Prime	dCTP only	25 ng	10 minutes	2×10^9	membrane hybridization
Ready-To-Go DNA labelling beads	Random-Prime	dCTP only	10 ng - 1 μg	5 minutes	2×10^9	membrane hybridization
Megaprime	Random-Prime	any dNTP	25 ng	10 minutes	2×10^9	membrane hybridization
Nick translation	Nick translation	any dNTP	1 μg	2-3 hours	2×10^9	Production of large amounts of probe
RNA labelling	SP6/T7 RNA polymerase	UTP	1 μg	1-2 hours	2×10^9	*in situ* hybridization
5'-end labelling	T4 Polynucleotide kinase	dATP	10 pmol ends	1 hour	5×10^6	membrane hybridization *in situ* hybridization
3'-end labelling	Terminal deoxynucleo-tidyl transferase	any dNTP	10 pmol ends	30-60 minutes	5×10^6	membrane hybridization *in situ* hybridization

Table 3 Guide to the properties of radioactive labelling systems

Rediprime II DNA Labelling System

Amersham Biosciences' premium radioactive labelling system consists of individually dispensed reaction mixes which are dried in the presence of a stabilizer and a dye to make labelling of probes easier. The system can be stored at 4°C or at room temperature ready for use.

Rediprime II reaction mixes have been formulated using an improved exonuclease free Klenow to give probes with specific activities of 1.9×10^9 dpm/mg or greater after 10 minutes incubation at 37°C with the majority of DNA substrates. When used with Redivue™ [^{32}P]dCTP, Rediprime II reactions can be set up and completed to produce a DNA probe ready for hybridization in less than 15 minutes.

Protocol Summary

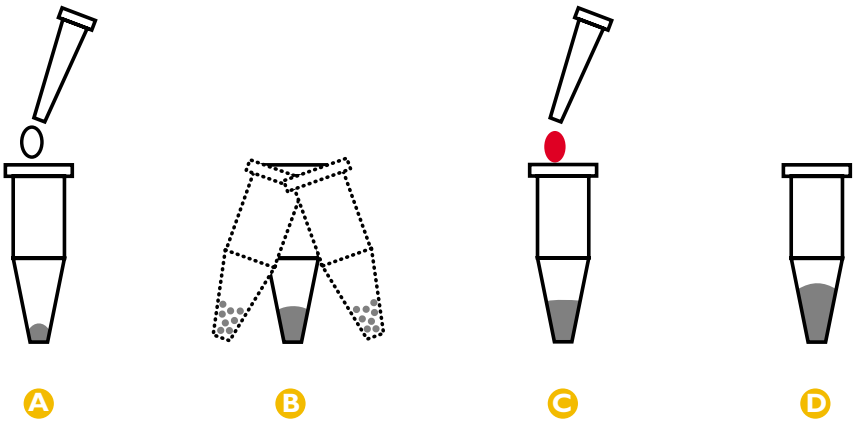

Figure 24
Schematic diagram of the Rediprime II protocol
(A) Add denatured template to a final volume of 45µl
(B) Flick tube and spin briefly
(C) Add 5ml Redivue [^{32}P]dCTP, pipette up and down and spin briefly
(D) Incubate for 10 minutes at 37°C

Recommended Applications

Labelling of DNA from a variety of sources to a high specific activity for use in Southern and Northern blot hybridizations. The system is designed for use with Redivue [^{32}P]dCTP with a specific activity of 110TBq/mmol, 3000 Ci/mmol.

Speed Quick and convenient protocol requires the addition of template and labelled nucleotide only.

Stability Ambient temperature stable, therefore can be stored at room temperature.

Flexibility DNA can be labelled in the presence of Low Melting Point agarose or restriction enzyme buffers.

Efficiency Each labelling mix can label up to 25 ng of DNA to a specific activity of >10^9 dpm/µg.

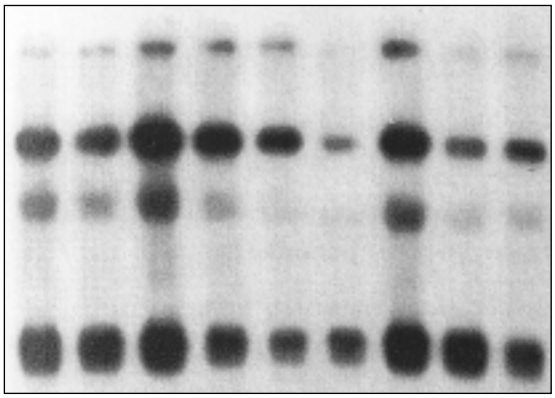

Figure 25
Northern blot probed with 3.8kb human EGFR cDNA fragments labelled with Rediprime. Result kindly supplied by J M Loughlin, Zeneca Pharmaceuticals, UK.

Ordering Information

Rediprime II DNA Labelling System	30 pre-mixed labelling reactions For use with radiolabelled dCTP	RPN1633
Rediprime II DNA Labelling System	60 pre-mixed labelling reactions For use with radiolabelled dCTP	RPN1634

Related products

Redivue [α-^{32}P]dCTP, 3000Ci/mmol	AA0005
Megaprime DNA Labelling System	RPN1604
Rapid-hyb Buffer	RPN1635
Hybond Nylon Membranes	see page 28

5'-End Labelling Kit

The 5'-End Labelling Kit exploits an optimized exchange buffer and T4 polynucleotide kinase to label oligonucleotides with or without a 5'-phosphate group. Labelling is complete within 30 minutes.

Recommended Applications

Oligonucleotides labelled in this way are suitable for use in high target amount applications. For example as probes or primers to track PCR products, or in gel-shift and fragment analysis assays.

Flexibility Very small fragments of both DNA and RNA can be labelled.

Reliability Location of the labelled group is known.

Protocol Summary

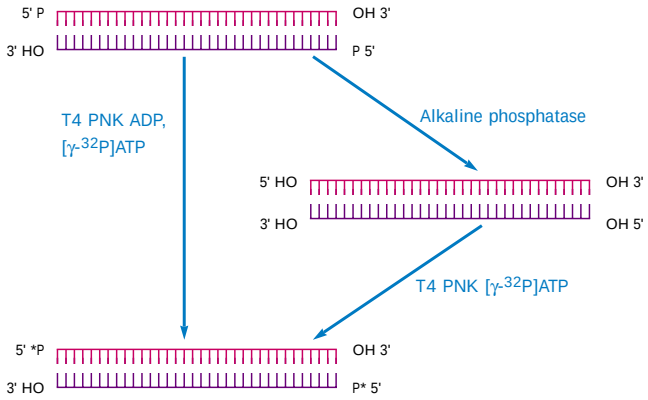

Figure 26
The 5' -End Labelling reaction using T4 polynucleotide kinase

Ordering Information

5'-End Labelling Kit	20 reactions	RPN1509

Related products

Redivue [γ-32P]dATP	AA0018
3'-End Labelling Kit	N4020

3'-End Labelling Kit

Terminal deoxynucleotidyl transferase adds deoxyribonucleotides onto the 3'-ends of DNA fragments. It can be used in conjunction with ^{32}P-, ^{33}P- ^{35}S- or ^{3}H-labelled nucleotides to label DNA for a variety of applications.

Protocol Summary

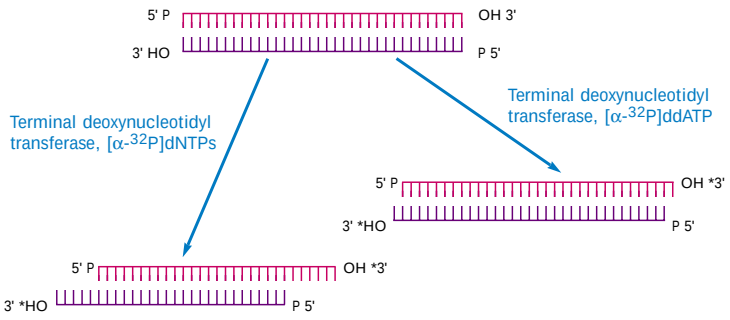

Figure 27
The 3' -End Labelling reaction using terminal deoxynucleotidyl transferase

Recommended Applications

One of the major applications for the 3'-End Labelling Kit is in the production of ^{32}P end-labelled oligonucleotide probes for screening applications involving colony, plaque or PCR clones. ^{35}S or ^{33}P end-labelled probes are used for *in situ* hybridization applications.

Flexibilty Template independent and all types of 3'-Ends can be labelled.

Reliability Location of the labelled group is known.

Ordering Information

3'-End Labelling Kit	20 reactions	N4020

Related products

Standard formulation radiolabelled nucleotides	RPN1509
5'-End Labelling Kit	RPN1509

Ready-To-Go PCR Beads

Ready-To-Go™ PCR Beads are pre-mixed, pre-dispensed, individual reactions designed for performing PCR amplification. Each dried room-temperature stable bead contains *Taq DNA polymerase, nucleotides and buffer, all optimized for standard PCR. Results are typically comparable with, or superior to, those using pre-formulated, aqueous 'master-mixes'.**

Figure 28
Ready-To-Go pre-dispensed reagent beads

Protocol Summary

Each pre-dispensed Ready-To-Go PCR Bead contains *Taq* DNA polymerase, nucleotides and buffer, all optimized for PCR.

Ready-To-Go PCR Beads are designed for 25 µl reactions. Simply add DNA template and primers and begin temperature cycling.

The beads are provided in either 0.5 ml or 0.2 ml tubes that are compatible with most thermocyclers. The 0.2 ml tubes come assembled in a convenient 96-well (8 x 12) plate format that allows individual strips of tubes to be easily removed. This flexibility allows the use of either the entire 96-well plate, strips of 8 or individual 0.2 ml tubes.

Recommended Applications

Ready-To-Go PCR Beads can be used with a variety of templates including genomic DNA, viral DNA, plasmid DNA and cDNA. They provide an excellent source of probe template for both radioactive and non-radioactive labelling techniques. In addition low levels of radioactivity may be directly incorporated for use in applications such as single stranded conformational polymorphism (SSCP) assays.

*For licence information see page 37

Convienience PCR components are provided in pre-formulated, single dose beads, reducing risk of pipetting errors and introducing contaminants. Pre-dispensed, individually packed PCR reactions, ensures a fresh reaction that dissolves quickly in a PCR compatible tube.

Consistency Application testing ensures each batch of beads delivers consistent results.

Compatability Each reaction is provided in a thin-walled 0.5 ml or 0.2ml tube, which fit directly into most thermocyclers.

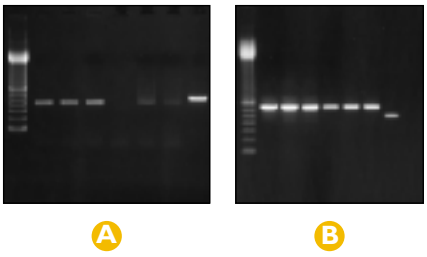

Figure 29
Comparison of Ready-To-Go Beads with conventional PCR mixtures for the amplification of genes form interferon-γ activated human aortic endothelial cells.
Panel (A) amplification of TGF-α.
Panel (B) amplification of β-actin.
M = 100 Base Pair Ladder, beads = Ready -To-Go PCR Beads; mix = conventional PCR mixture; + = control reaction supplied with PCR beads.

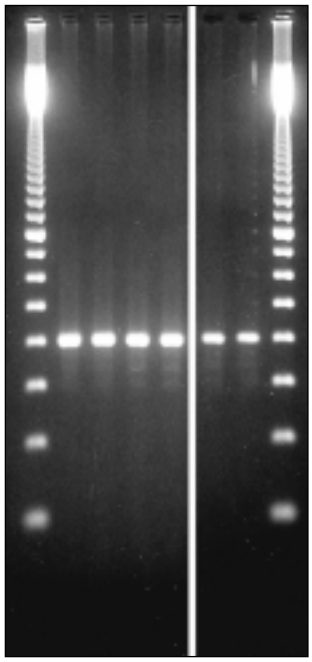

Figure 30
PCR results comparing performance of Ready-To-Go PCR Beads with pre-formulated, aqueous 'master mixes'. Both the PCR Beads and the 'master-mixes' contained all components for PCR except template and primer. 100 ng of human genomic template and primers specific for human aromatase, a single-copy gene, were added to each reaction to a final volume of 25 µl. Reactions were subjected to 35 cycles of : 95°C for 1 minute, 55°C for 1 minute; 72°C for 2 minutes. An equal volume of each reaction was loaded onto an agarose gel.
M = 100 base-pair ladder. Data courtesy of Denise Garvin.

Ordering Information

Ready-To-Go PCR Beads (0.5 ml tubes)	100 reactions	27-9555-01
Ready-To-Go-PCR Beads (0.2 ml tubes/plate)	96 reactions	27-9553-01
Ready-To-Go-PCR Beads (0.2 ml tubes/plate)	5 x 96 reactions	27-9553-02

Related products

Ready-To-Go DNA Labelling Beads	27-9240-01

RNA Labelling Kit

The RNA Labelling Kit has been designed to give high-specific activity single-stranded RNA probes *in vitro*. This kit contains RNase-free DNase I for template removal and pre-prepared dithiothreitol (DTT) for added convenience. The kit comes with SP6 and T7 RNA polymerases and can also be used with T3 RNA polymerase, which must be purchased separately.

Figure 31
Beta work tank

Recommended Applications

For the generation of high specific activity RNA probes for membrane hybridization and *in situ* applications. Also full length RNA probes for RNase protection studies.

Sensitivity RNA probes show higher sensitivity than equivalent nick-translated probes in both Northern and *in situ* hybridizations.

Stability RNA:RNA hybrids are more stable than DNA:DNA or DNA:RNA hybrids, due to avoidance of reannealing and stronger hydrogen bonding.

Specificity Removal of non-specific bound probe by treatment with RNase A which is very specific for single-stranded RNA.

Ordering Information

RNA Labelling Kit	20 labelling reactions up to 1 µg	RPN3100

Related products

Redivue [α-^{32}P]UTP	AA0003
T3 RNA Polymerase	E7005 1Y

Rapid-hyb Buffer

Rapid-hyb™ Buffer is a rate enhancing hybridization buffer for rapid hybridization of radiolabelled nucleic acid probes to membrane-bound targets. In some Northern blotting experiments Rapid-hyb Buffer contributed to a five fold improvement in sensitivity.

Recommended Applications

Rapid-hyb Buffer is optimized for use in a wide range of applications, including Southern, Northern, dot/slot blots and colony/plaque lifts.

Speed Single-copy gene detection is possible after only a 2 hour hybridization with ^{32}P-labelled probes.

High signal to noise ratio Inclusion of chemical blocking agents ensures low backgrounds.

Stability Stores at room temperature – ready to use without addition of carrier DNA.

Compatibility Compatible with DNA, RNA and oligonucleotide probes.

Versatility A wide range of hybridization temperatures (42°C – 70°C) can be used for optimal results.

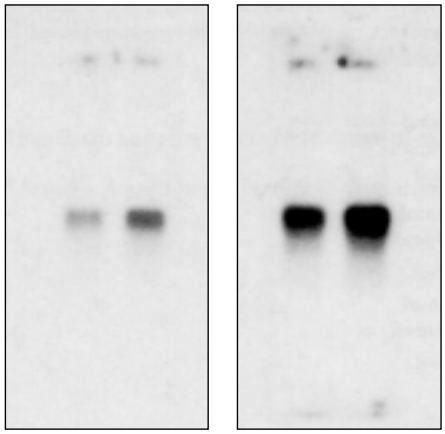

Figure 32
Northern blot analysis using:
(A) Standard hybridization buffer (B) Rapid-hyb buffer
Northern blots of HeLa cell total RNA (0.5 µg loadings). Linearized pHSP70 probe labelled with (α-^{32}P)dCTP using megaprime labelling system. Hybridizations were at 65°C for 1 hour using a probe concentration of 2 ng/ml. Exposure to Hyperfilm MP overnight.

Ordering Information

Rapid-hyb Buffer	125 ml	RPN1635
Rapid-hyb Buffer	500 ml	RPN1636

Related products

Rediprime II DNA Labelling System	RPN1633
5'-End Labelling Kit	RPN1509
3'-End Labelling Kit	N4020

Redivue Nucleotides

Redivue™ ^{32}P, ^{33}P and ^{35}S nucleotides contain a novel red dye and stabilizer solution, providing advantages in both handling and storage. Redivue is much easier to use being clearly visible in your pipette tip and in reaction mixes down to 1 µl in 50 µl. Redivue nucleotides can be used straight from the refrigerator in liquid form which saves time and avoids freeze thaw cycles.

Figure 34
Redivue nucleotides can make experiments quicker to set up

Recommended Applications

Redivue nucleotides can be substituted directly for standard formulation products in the majority of molecular biology applications.

Ease of use Addition of an intense red dye improves visibility and handling characteristics.

Stability Stabilized format allows storage at 4°C in a convenient liquid form.

Compatibility Exhaustive testing of each Redivue product ensures equivalent performance to standard format radiolabelled nucleotides.

Consistency Elimination of repeated freeze thaw cycles ensuring consistent performance.

Figure 33
Use straight from the fridge. Dispensing is simpler with easy to use Redivue nucleotides

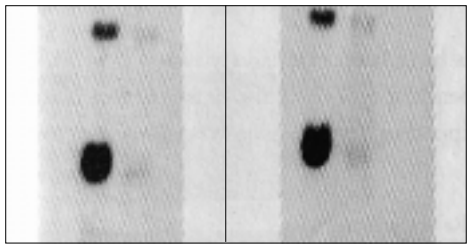

Figure 35
Detection of B-myb mRNA by Northern hybridization using Redivue or standard [α-^{32}P]dCTP
Results supplied by Dr E Lam, Ludwig Institute for Cancer Research, London

Ordering Information

Redivue Nucleotides

[α-^{32}P]dATP	~220 TBq/mmol, ~6000 Ci/mmol	AA0074
[α-^{32}P]dATP	~110 TBq/mmol, ~3000 Ci/mmol	AA0004
[α-^{32}P]dATP	~30 TBq/mmol, ~800 Ci/mmol	AA0084
[α-^{32}P]dATP	~15 TBq/mmol, ~400 Ci/mmol	AA0064
[α-^{32}P]dCTP	~220 TBq/mmol, ~6000 Ci/mmol	AA0075
[α-^{32}P]dCTP	~110 TBq/mmol, ~3000 Ci/mmol	AA0005
[α-^{32}P]dCTP	~30 TBq/mmol, ~800 Ci/mmol	AA0085
[α-^{32}P]dCTP	~15 TBq/mmol, ~400 Ci/mmol	AA0065
[α-^{32}P]dGTP	~110 TBq/mmol, ~3000 Ci/mmol	AA0006
[α-^{32}P]dGTP	~30 TBq/mmol, ~800 Ci/mmol	AA0086
[α-^{32}P]dGTP	~15 TBq/mmol, ~400 Ci/mmol	AA0066
[α-^{32}P]dTTP	~110 TBq/mmol, ~3000 Ci/mmol	AA0007
[α-^{32}P]dTTP	~30 TBq/mmol, ~800 Ci/mmol	AA0087
[α-^{32}P]dTTP	~15 TBq/mmol, ~400 Ci/mmol	AA0067
[α-^{32}P]dUTP	~110 TBq/mmol, ~3000 Ci/mmol	AA0003
[γ-^{32}P]dATP	> 185 TBq/mmol, >5000 Ci/mmol	AA0018
[γ-^{32}P]dATP	~110 TBq/mmol, ~3000 Ci/mmol	AA0068
[γ-^{33}P]dATP	≥ 92.5 TBq/mmol, ≥ 2500 Ci/mmol	AH9968
[α-^{33}P]dATP	≥ 92.5 TBq/mmol, ≥ 2500 Ci/mmol	AH9904
[α-^{33}P]dCTP	≥ 92.5 TBq/mmol, ≥ 2500 Ci/mmol	AH9905
[α-^{33}P]dUTP	≥ 92.5 TBq/mmol, ≥ 2500 Ci/mmol	AH9903
[^{35}S]dATPαS	> 37 TBq/mmol, >1000 Ci/mmol	AG1000
[^{35}S]dATPαS	~22 TBq/mmol, ~600 Ci/mmol	AG1001
[^{35}S]dATPαS	~15 TBq/mmol, ~400 Ci/mmol	AG1002
[^{35}S]dCTPαS	> 37 TBq/mmol, >1000 Ci/mmol	AG0135
[^{35}S]dCTPαS	~22 TBq/mmol, ~600 Ci/mmol	AG0035
[^{35}S]dCTPαS	~15 TBq/mmol, ~400 Ci/mmol	AG0025

Related products

Rediprime II DNA Labelling System	RPN1633
Ready-To-Go DNA Labelling Beads (dCTP)	27-9240-01
5'-End Labelling Kit	RPN1509
3'-End Labelling Kit	RPN4020
RNA Labelling Kit	RPN3100
Ready-To-Go PCR Beads	27-9555-01

Hybond Membranes for Nucleic Acid Blotting

Hybond™ membranes are available to support all major nucleic acid blotting applications.

Rely on Amersham Biosciences' technical experience in all types of blotting for clear guidance on the choice of membrane best suited for your application.

Individual technical advice is available on all aspects of blotting and hybridization via e:mail at

Hybond_Help@eu.amershambiosciences.com

Choosing the correct membrane

The following table is designed to help in the choice of the best possible membrane for each application

Application	Method	Recommended membrane
Southern blotting	Radioactive detection	Hybond-XL
	Non-radioactive detection	Hybond-N+
	Alkali blotting	Hybond-XL / Hybond-N+
	Low volume hybridizations	Hybond-NX
	Rapid-hyb Buffer	Hybond-XL
	Fingerprinting	Hybond-Nfp
Northern blotting	Radioactive detection	Hybond-XL
	Non-radioactive detection	Hybond-N+
	Low volume hybridizations	Hybond-NX
	Rapid-hyb Buffer	Hybond-XL
Dot and slot blots	Radioactive detection	Hybond-XL
	Non-radioactive detection	Hybond-N+
Colony and plaque blots	Radioactive detection	Hybond-XL
	Non-radioactive detection	Hybond-N+

Table 4 Choosing the correct membrane

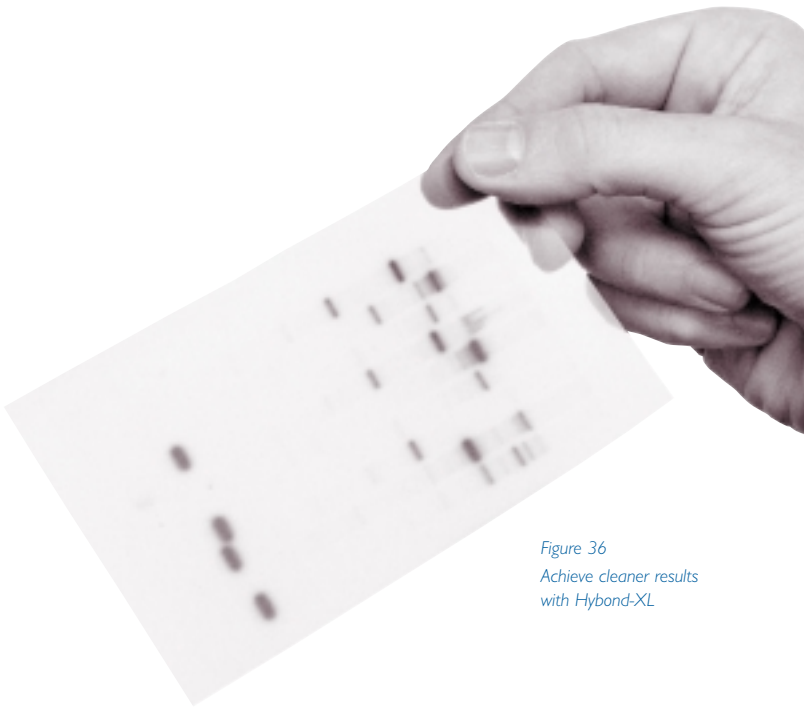

Figure 36
Achieve cleaner results with Hybond-XL

Hybond-XL Hybond-XL is a charged nylon membrane designed exclusively for radioactive nucleic acid transfer applications to achieve an improved signal-to-noise ratio. It has a greater concentration and a more even distribution of positive charge than other charged nylon membranes. Hybond-XL produces excellent results with a wide variety of applications and target/probe combinations. This membrane retains all the advantages of other nylon membranes such as high nucleic acid binding capacity and high tensile strength.

Hybond-N+ Hybond-N+ is a positively charged nylon membrane which yields excellent sensitivity in both alkali blotting and conventional Southerns. Nucleic acid samples may be fixed by simple alkali treatment or alkali blotting rather than UV exposure or baking, though UV fixation is recommended for maximum reproducibility.

Hybond-N Hybond-N is a neutral nylon membrane capable of high sensitivity in DNA and RNA blotting. It is a strong supported membrane that is inherently hydrophilic and requires no pre-wetting. It is easy to quickly crosslink nucleic acids to nylon using UV light. This makes Hybond-N ideal for any standard radioactive Southern or Northern blotting procedure, except for customers using low hybridization volumes where Hybond-NX is recommended.

Hybond-NX Hybond-NX is a neutral nylon membrane with all the properties of Hybond-N but has been specially developed for use with high throughput applications where low hybridization buffer volumes are used. Hybond-NX gives cleaner background than Hybond-N when low buffer volumes are used.

Hybond-Nfp Hybond-Nfp is a neutral nylon membrane with all of the properties of Hybond-N, it was launched in response to the needs of researchers performing DNA fingerprinting. These customers preferred to validate a sample of a particular batch in their application before ordering the rest of the batch. Validation in this demanding technique can take up to 2 months, therefore to remove this inconvenience we offer Hybond-Nfp which is pre-validated by independent third party DNA fingerprinting experts.

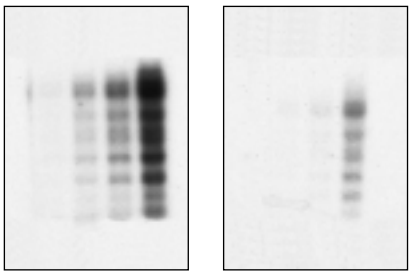

Figure 37
Human genomic Southern blot using:
A) Hybond-XL B) leading competitor membrane
Human genomic DNA, digested with Hind *III 2, 1, 0.5, 0.1 µg loadings. GAPDH random prime [^{32}P] labelled probe, 2 x 10^{6} cps/ml. Church hybridization buffer. Overnight exposure to Hyperfilm MP.*

Autoradiography Films

Amersham Biosciences' expertise in the labelling and detection of biological molecules supports a range of autoradiography products designed specifically for life science applications.

This range includes the market leading Kodak™ X-Omat™ AR film and the high speed BioMax™ MR and BioMax MS films which utilize Kodak T-grain emulsion technology.

Use the following table to help choose of the best possible film and exposure conditions for your application.

Application	Result required	Label	Method	Film
Southern/Northern blots Colony/plaque blots	High speed and sensitivity Accurate quantitation	^{32}P	Pre-flash with screens at -70°C	Hyperfilm MP
	Maximum speed and sensitivity	^{32}P	Screens at -70°C	BioMax MS
	Optimum resolution	^{33}P/^{35}S	Direct autoradiography BioMax MR	Hyperfilm βmax
	Non-radioactive	ECL	Pre-flash and direct detection	Hyperfilm ECL
Slot blots	High speed and sensitivity accurate quantitation	^{32}P	Pre-flash with screens at -70°C	Hyperfilm MP
	Maximum speed and sensitivity	^{32}P	Screens at -70°C	BioMax MS
	non-radioactive	ECL	Pre-flash and direct detection	Hyperfilm ECL
Dideoxy sequencing	High speed	^{32}P	Direct autoradiography	Hyperfilm MP BioMax MR
	Optimum resolution and maximum speed for difficult sequences	^{35}S	Direct autoradiography	Biomax MR
	Good resolution and speed for routine work	^{35}S	Direct autoradiography	Hyperfilm MP
Cycle sequencing	Optimum resolution and maximum speed for difficult sequences	^{33}P	Direct autoradiography	BioMax MR
	Good resolution and speed for routine work	^{33}P	Direct autoradiography	Hyperfilm MP
***In situ* hybridization**	Macroscale *in situ* optimization of parameters	^{32}P	Direct autoradiography	Hyperfilm MP

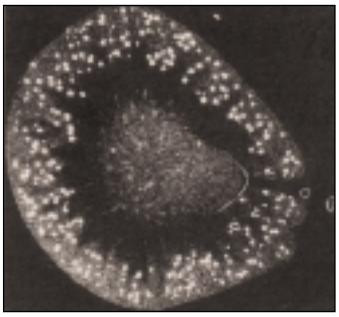

Figure 38 Rat kidney.
Total ^{125}I ANP binding showing renal blood vessels, smooth muscle, renal papilla, glomeruli in cortex and the inner medulla. Reversed image from Hyperfilm ^{3}H.
Photograph courtesy of John Wharton and Richard Rutherford. Department of Histochemistry, Royal Postgraduate School, London.

Hyperfilm MP An excellent multipurpose film. The clear base gives high contrast and good resolution while its double-sided construction and compatibility with intensifying screens means that it performs well in blotting applications.

Hyperfilm β-max A specialized film with high sensitivity, high resolution and a good linear range.

Hyperfilm ECL The recommended film for chemiluminescent applications. The clear base gives high contrast and good resolution while its sensitivity profile matches the emission spectrum of ECL and other chemiluminescent systems.

BioMax MR An excellent film for the direct autoradiography of ^{35}S, ^{14}C and ^{33}P. This clear, single-sided film gives high resolution and contrast and the special T-grain technology makes it twice as fast as X-Omat AR with weak emitters.

BioMax MS This film and screen combination offers exceptional sensitivity in ^{32}P and ^{125}I applications. The high performance film of choice for radioactive blotting.

X-Omat AR A general-purpose film for use with all commonly used isotopes. Coated with emulsion on both sides for both direct exposure and use with intensifying screens.

Related autoradiography accessories

Hypercassette™ autoradiography cassettes
Hyperprocessor™ automatic film processor
Hyperscreen™ intensifying screens
Hypertorch™
Kodak BioMax TranScreen intensifying screens
Safelights
Sensitize™ preflash unit
Trackertape™

Ordering information

For further information please request 'The Complete Autoradiography Product Guide'.

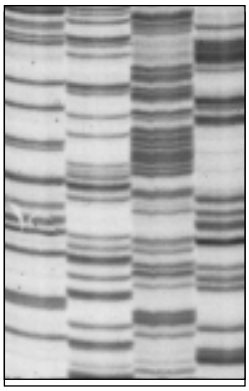

Figure 39
Cycle sequencing with ^{33}P labelled primers. Detected on Hyperfilm MP.

Amersham Biosciences Scanners

Storm

The Amersham Biosciences™ Storm™ imaging system for gel, blot and macroarray analysis combines proven storage phosphor imaging technology for radioisotope detection with fluorescence and chemifluorescence imaging for non-radioactive detection. Researchers can make the move to non-radioactive gel and blot analysis without sacrificing conventional radioisotope techniques. Storm scans storage phosphor screens and fluorescent and chemifluorescent gels and blots in a large format 35 x 43cm scanning area or smaller. ImageQuant™ software for Windows NT™ or Macintosh™ is included. ECF detection reagents provide optimized chemifluorescent detection of Southern and Northern blots on the Storm system.

Place your storage phosphor screen, gel or blot on the sample area, and point and click to start your scan. The Storm system uses patented Variable Mode Imaging (VMI) architecture to activate the correct optical components for storage phosphor autoradiography, direct fluorescence or chemifluorescence.

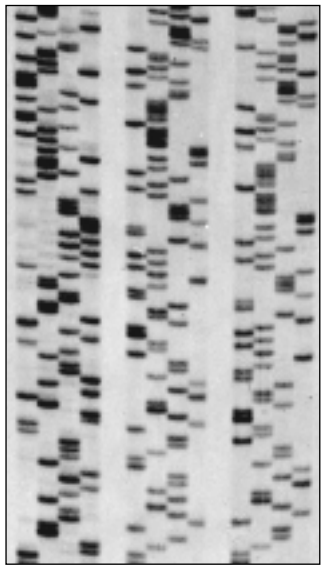

Figure 41
Large samples fit on the Storm systems 35 x 43cm scan area. Storm offers the high resolution needed for DNA base identification.

Figure 40
Storm imaging system in use

PhosphorImager SI

The Amersham Biosciences PhosphorImager™ SI offers advanced technology for autoradiography in a compact design. Storage phosphor screens capture images produced by x-rays and radioisotopes from radiolabelled blots, gels, arrays, TLC plates or tissues. Exposure times are reduced by at least 90% relative to an equivalent exposure to autoradiography film and the PhosphorImager SI can accurately quantitate both strong and weak signals from the same exposure. The PhosphorImager SI offers significant advantages in data throughput and analysis.

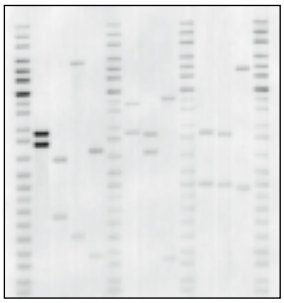

Figure 43
Southern blotting with chemifluorescence. Bands were visualized by chemifluorescence in this Southern blot of human genomic DNA.

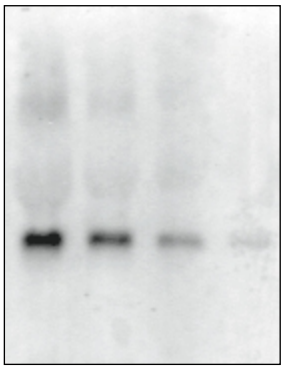

Figure 44
Northern blotting with chemifluorescence. A fluoresceinated cDNA probe was used to identify p53 mRNA in A172 glioblastoma cells. Bands were visualized by chemifluorescence.

FluorImager

FluorImager™ systems are multi-purpose fluorescent laser scanners for multiple colour analysis of gels, blots, arrays, TLC, and microplates. Samples are read directly, with no drying or exposure step required. Software for instrument control, quantitative image analysis and printing is included.

Figure 42
FluorImager 595 provides fast, fluorescent gel and blot analysis

Accessory products

UV Crosslinker

The Amersham Biosciences™ UV Crosslinker reduces the time required to fix nucleic acids on membranes from hours to less than a minute.

UV-fixed samples give consistently better hybridization signals than baked samples, especially after repeated re-probings. Nucleic acids can be UV-crosslinked to nylon, nitrocellulose and supported nitrocellulose membranes.

Reliability Short-wave UV light crosslinks DNA or RNA securely to membranes for repeated re-probings.

Accuracy Microprocessor controlled for accurate and reproducible UV dosage.

Convenience Keypad selection of pre-programmed or user-set energy or time exposures.

Flexibility Spacious chamber accepts membranes up to 25 x 30cm, but compact footprint requires only 35 x 40cm of bench space.

Figure 45
The Amersham Biosciences UV Crosslinker

Ordering Information

Ultraviolet Crosslinker 115 V 60 Hz Includes five 8 W short-wave UV lamps	80-6222-31
Ultraviolet Crosslinker 230 V 50 Hz Includes five 8 W short-wave UV lamps	80-6222-50
8 W, 254 nm replacement UV lamp	80-6223-64

Vacuum Blotting Units

Vacuum blotting is a quick and reproducible method for transferring nucleic acid to membranes. The specially designed vacuum pump draws the DNA/RNA through the agarose gel and onto the membrane, therefore reducing transfer times to less than an hour.

Speed Rapidly transfer DNA or RNA from agarose gels up to 20 x 30 cm in size.

Efficiency Efficient and reproducible transfers improve blotting results.

Convenience Minimize gel handling by performing all pre-transfer steps in the unit.

Accuracy Use with VacuGene™ XL blotting pump ensures precise vacuum control.

Ordering Information

VacuGene XL Vacuum Blotting System	13-2223-01
VacuGene XL Vacuum Blotting Unit	80-1266-24
VacuGene XL Vacuum Blotting Pump 110 V	80-1265-14
VacuGene XL Vacuum Blotting Pump 220 V	80-1265-15

Submarine Electrophoresis Units

Electrophoresis is an integral part of many molecular biology techniques, and as such requires equipment which is convenient, robust and adaptable.

The comprehensive range of submarine electrophoresis equipment from Amersham Biosciences can accommodate virtually any need for gel size, buffer volume and cooling requirement.

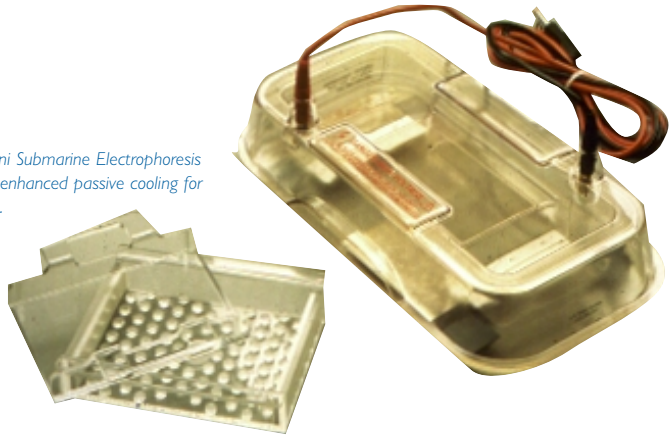

Figure 47
HE 33 Mini Submarine Electrophoresis Unit gives enhanced passive cooling for faster runs.

Amersham Biosciences HE 33 Mini Submarine Electrophoresis Unit

Coolant filled buffer chamber base provides passive cooling, allowing gels to be run at high voltages. 7 x 10cm agarose gels can be run in less than 20 minutes.

Amersham Biosciences HE 99X Max Submarine Electrophoresis Unit

Versatile and durable, 3 different gel lengths and 12 different comb sizes allows customization of gel configuration. Gels are cast without the need for tape.

Amersham Biosciences HE 100 SuperSub™ Submarine Electrophoresis Unit

High performance, large format electrophoresis unit. Contains a built-in magnetic stir bar for buffer circulation and an integral heat exchanger allowing high voltage or extended runs.

EPS 301 Power Supply

Flexible, economical power supply for submarine electrophoresis units at up to 300V or 400mA with 80W power. This versatile unit also handles vertical gels and some electrophoretic blotting.

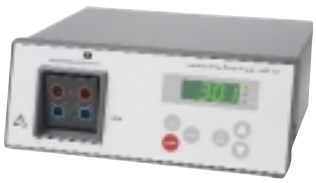

Figure 46
EPS301 Power Supply

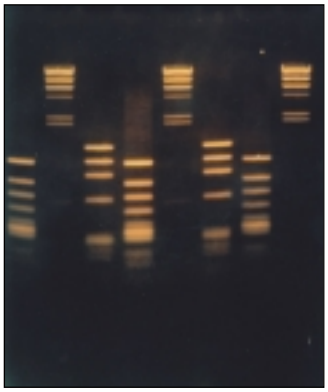

Figure 48
Separation of DNA size markers in HE 33 Submarine Unit. Lanes 1, 4, 7: ϕX-174 RF DNA-Hind II digest; lanes 2, 5, 8: lambda DNA-Hind III digest; lanes 3, 6: ϕX-174 RF DNA-Hae III digest. Markers were separated for 30 minutes at 200V in a 1% agarose gel.

Ordering Information

Amersham Biosciences HE 33 Mini Submarine Electrophoresis Unit, complete	80-6052-45
Amersham Biosciences HE 99 Max Submarine Electrophoresis Unit, complete	80-6061-57
Amersham Biosciences HE 100 SuperSub Electrophoresis Unit, complete	80-6043-71
EPS 301 Power Supply	18-1130-01

Accessory products

Hybridization Oven/Shaker

The Hybridization Oven/Shaker rigidly controls the hybridization temperature and frequency of agitation, thus ensuring that stringency and washing conditions are maintained. Developed for use with both radioactive and non-radioactive labelling systems. A choice of hybridization modes is available by use of a variable speed rotisserie or platform shaker.

Economy Reduced hybridization buffer volumes minimizes probe usage.

Safety Acrylic/polycarbonate door minimizes exposure to beta radiation.

Versatility Interchangeable rotisserie and platform shaker.

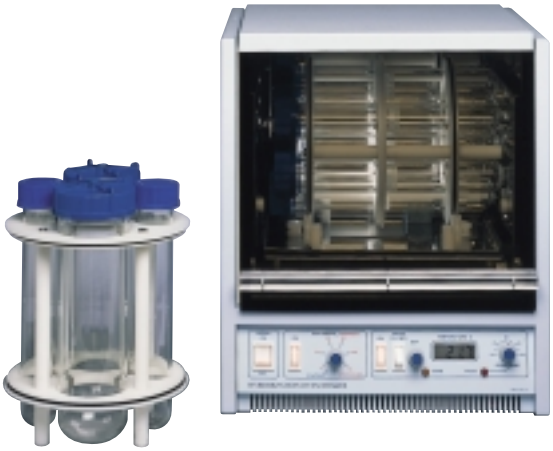

Figure 49
Hybridization Oven Shaker

Ordering Information

Hybridization Oven/Shaker 220/240 V 50 Hz	RPN2510
Hybridization Oven/Shaker 110/115 V 60 Hz	RPN2511
Hybridization Oven/Shaker 100 V 50/60 Hz	RPN2512

Electrophoresis Chemicals

In gel electrophoresis the quality of the chemicals used for gel casting and gel staining is critical to obtain good results.

Ethidium Bromide Widely used for detecting DNA and RNA, ethidium bromide binds to double-stranded regions to form UV fluorescent complexes. As little as 50 ng of DNA can be detected with concentrations of 0.5 – 1.0 µg/ml of stain.

Agarose Agarose with low electroendosmosis is recommended for most DNA and RNA applications. To ensure maximum enzyme efficiency, a highly purified Agarose NA can be used. For preparative work, Agarose Prep with low gelling temperature should be used.

Ordering Information

Ethidium Bromide Solution, 10 mg/ml	17-1328-01
Agarose NA, 100 g	17-0554-02
Agarose Prep, 50 g	80-1103-07

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