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

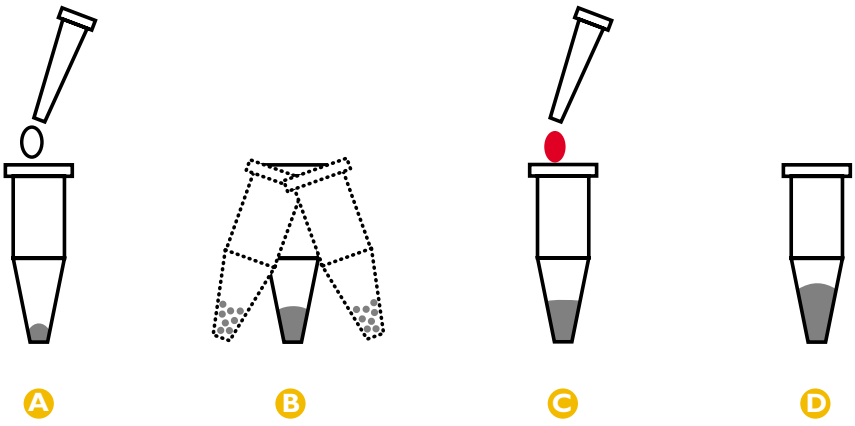

Figure 24
Schematic diagram of the Rediprime II protocol
(A) Add denatured template to a final volume of 45µl
(B) Flick tube and spin briefly
(C) Add 5ml Redivue [^{32}P]dCTP, pipette up and down and spin briefly
(D) Incubate for 10 minutes at 37°C

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.

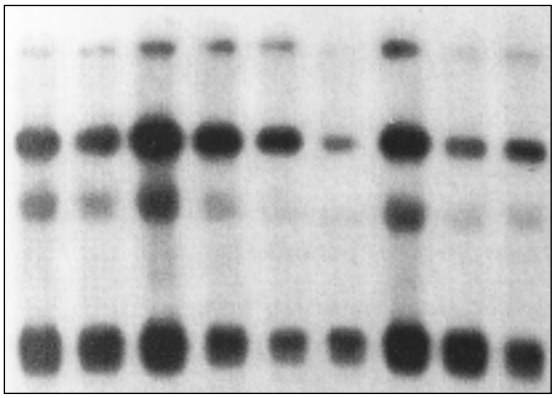

Figure 25
Northern blot probed with 3.8kb human EGFR cDNA fragments labelled with Rediprime. Result kindly supplied by J M Loughlin, Zeneca Pharmaceuticals, UK.

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

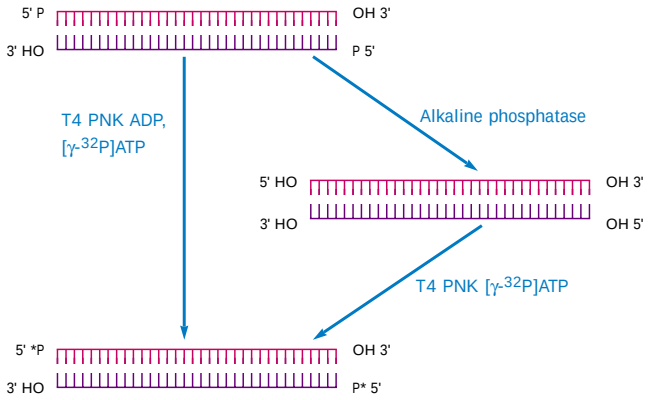

Figure 26
The 5' -End Labelling reaction using T4 polynucleotide kinase

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

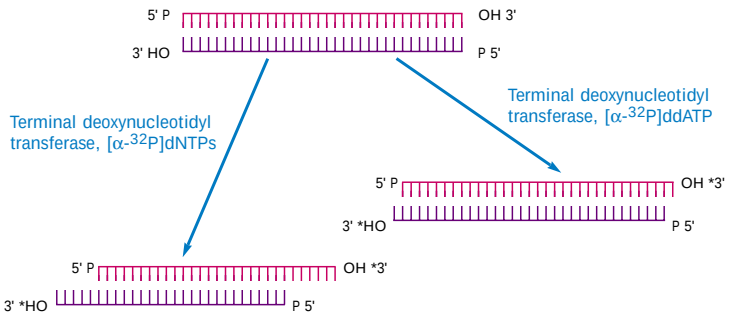

Figure 27
The 3' -End Labelling reaction using terminal deoxynucleotidyl transferase

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.

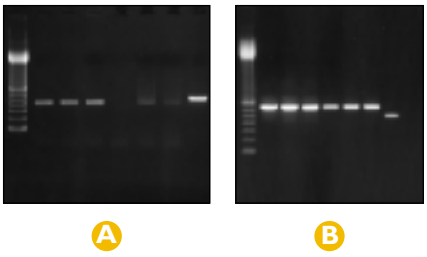

Figure 29
Comparison of Ready-To-Go Beads with conventional PCR mixtures for the amplification of genes form interferon-γ activated human aortic endothelial cells.
Panel (A) amplification of TGF-α.
Panel (B) amplification of β-actin.
M = 100 Base Pair Ladder, beads = Ready -To-Go PCR Beads; mix = conventional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

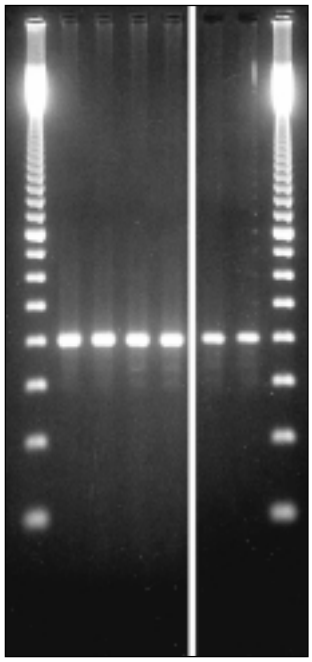

Figure 30
PCR results comparing performance of Ready-To-Go PCR Beads with pre-formulated, aqueous 'master mixes'. Both the PCR Beads and the 'master-mixes' contained all components for PCR except template and primer. 100 ng of human genomic template and primers specific for human aromatase, a single-copy 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.

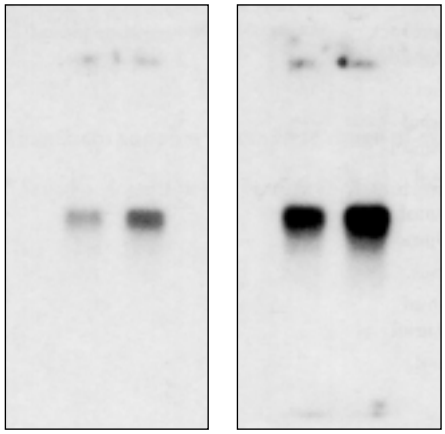

Figure 32
Northern blot analysis using:
(A) Standard hybridization buffer (B) Rapid-hyb buffer
Northern blots of HeLa cell total RNA (0.5 µg loadings). Linearized pHSP70 probe labelled with (α-^{32}P)dCTP using megaprime labelling system. Hybridizations were at 65°C for 1 hour using a probe concentration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

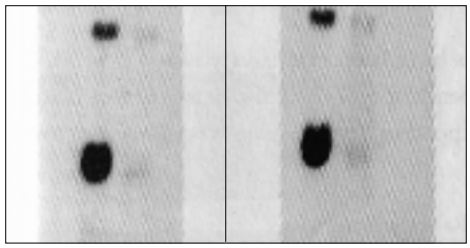

Figure 35
Detection of B-myb mRNA by Northern hybridization using Redivue or standard [α-^{32}P]dCTP
Results supplied by Dr E Lam, Ludwig Institute for Cancer Research, London