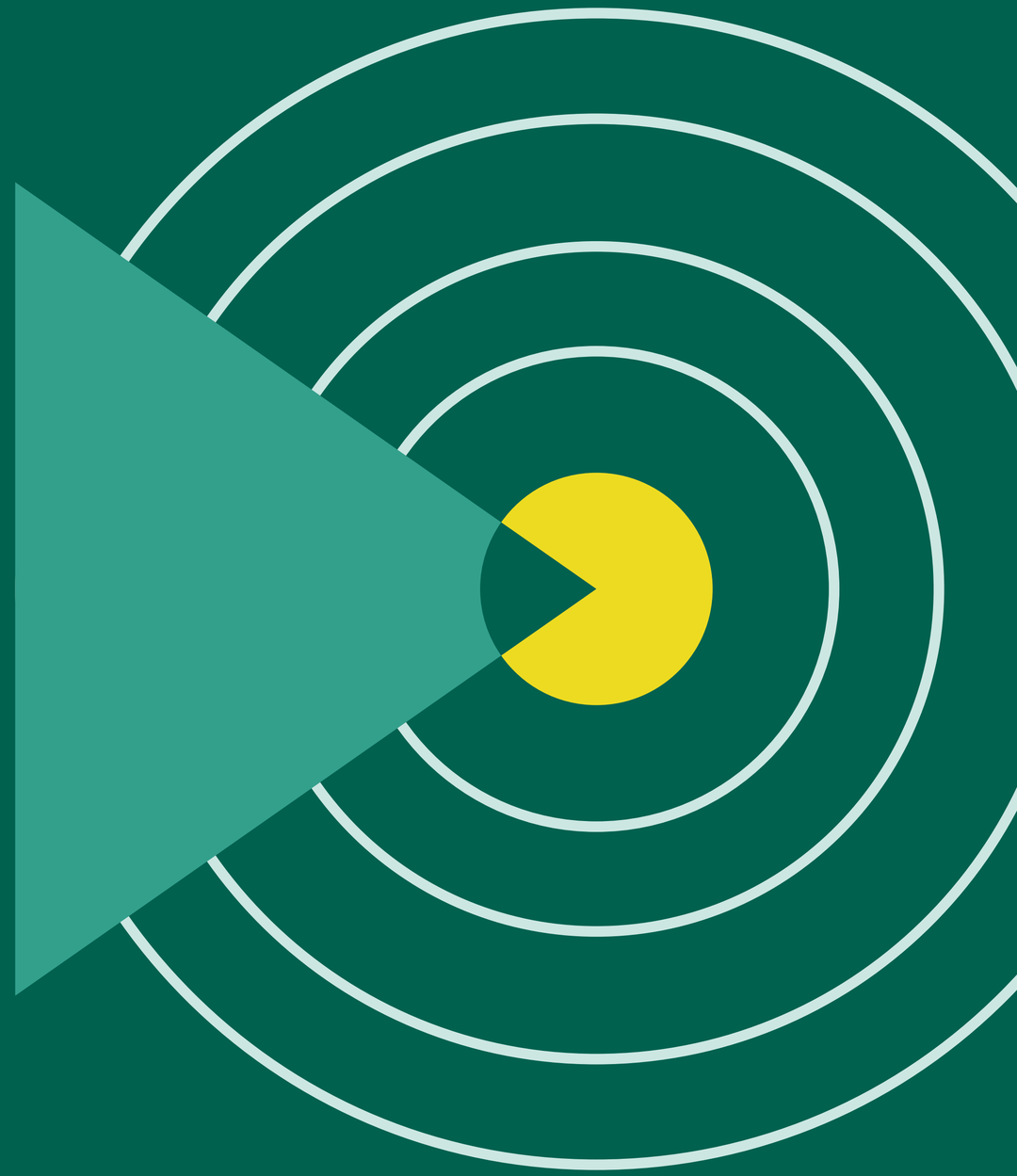
Product guide

Nucleic acid labeling and detection



cytiva

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Introduction

Researchers into DNA, RNA and oligonucleotides can choose from a wide range of labeling tools and techniques. With so many options, 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 labeling has the advantage of high sensitivity and extremely robust protocols, but safety and longterm probe storage are serious considerations.

The aim of this guide is to give an overall introduction to the different radioactive and non-radioactive methods available, highlighting their advantages and drawbacks. We will then present individual products in more detail to help you choose the most appropriate method for your application.

Table 1. Factors affecting the choice of labeling and detection system

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

Choosing a non-radioactive labeling system

Labeling

Non-radioactive labeling methods include direct and indirect approaches.

Direct labeling

Direct labeling using alkaline phosphatase offers significant advantages in speed and convenience over indirect systems without compromising sensitivity.

With direct labeling 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 labeling methods eliminate the need for an antibody conjugate incubation and the associated blocking and multiple washing steps.

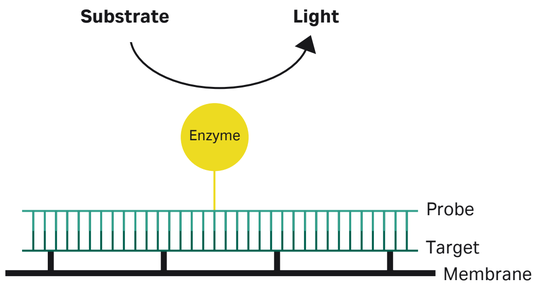

Fig 1. Direct labeling.

Indirect labeling

Indirect labeling involves the introduction into the probe of nucleotides tagged with a hapten or 'reporter' molecule. Cytiva kits use fluorescein as the hapten molecule. The nucleotide analogues are readily incorporated into the probe by standard labeling methods, and hybridization and stringency washing are carried out under standard conditions. Labeled 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 catalyzed detection, using the appropriate substrate.

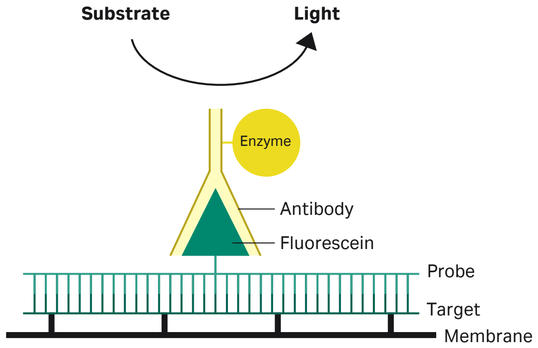

Fig 2. Indirect labeling.

Non-radioactive labeling systems offer the following benefits:

- **Speed**
  Exposure times 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 labeled probes are stable for at least 6 months, removing the need to label probes immediately before use.
- **Safety and ease of handling**
  Eliminates handling and waste regulatory issues associated with the use of radioactivity.

Consider both the labeling and detection procedures to make the correct choice of non-radioactive system.

Detection

Choice of detection method will depend on the enzyme used in the labeling 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 labeled 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 labeled either directly or indirectly with alkaline phosphatase. It is well-suited 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. ECF detection can be used to detect probes which have been either directly or indirectly labeled with alkaline phosphatase and is particularly suitable for applications where quantification is important.

Table 2. Guide to the properties of Cytiva non-radioactive labeling and detection systems

	AlkPhos Direct	ECL™ Direct
Sensitivity	0.06 pg	0.5 pg
Time from hybridization to detection	1 hour	1 hour
Duration of light output	5 days	1–2 hours
Strip before re-probing	yes	no
Quantification	no	no
Recommended application	Single copy Southern and northern blot	High target applications e.g. colony/plaques

AlkPhos Direct Labeling and Detection System

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

Labeling 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.

Protocol summary

Probe labeling

Denatured or single-stranded DNA or RNA probe is mixed with the labeling 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.

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 prehybridization step, hybridization with probe is performed overnight at the required temperature. For higher target amounts, a 2 to 4 hour hybridization may be sufficient.

Detection

Chemiluminescence with CDP-*Star*™

Hybridized blots are detected with CDP-*Star*™ Detection 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 60 fg can be achieved using CDP-*Star*™.

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.

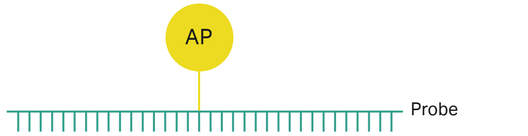

Fig 3. Probe labeling.

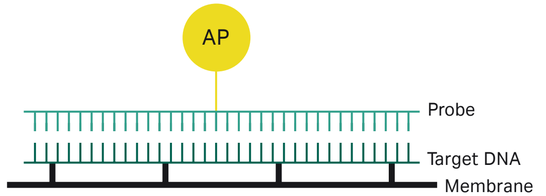

Fig 4. Hybridization.

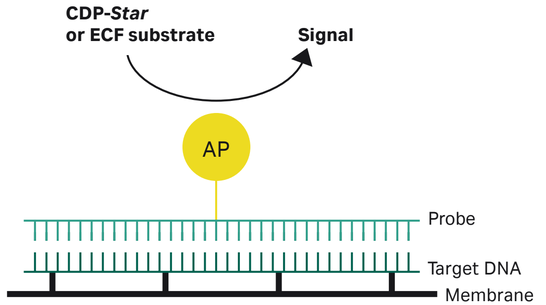

Fig 5. Detection.

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 labeled 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. This combination is well-suited 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 labeling oligonucleotides, an optimized protocol has been developed which is capable of labeling probes down to 17 base pairs in length.

Dot and slot blots can be quantified by using ECF detection in conjunction with AlkPhos Direct. Analyzing results on a fluorescence scanner allows accurate quantification and analysis of results.

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

Consistency: Quality control on each batch ensures consistent performance and provides confidence in probe labeling 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: Labeling 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 labeled efficiently and uniformly with enzyme.

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

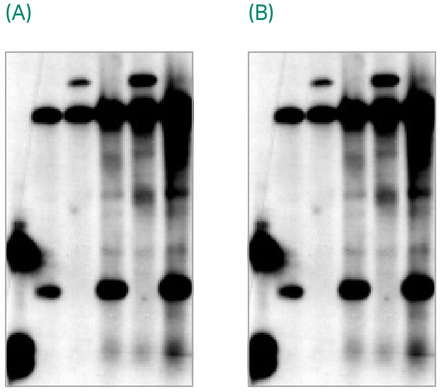

Fig 6. Southern blot. Cosmid DNA digested with Not I and EcoR I, probed with a 1.1 kb probe labeled with (A) AlkPhos Direct and (B) a competitor's hapten-based system. Courtesy of Janet Bartels, Yale University.

Fig 7. Northern blot (Hybond™-N+ nylon membrane) 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, and (C) CDP-*Star*™ re-applied after re-probing.

(A) (B)

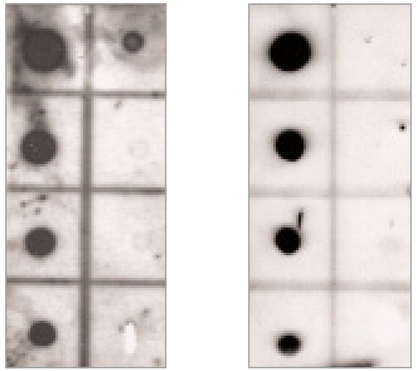

Fig 8. Dot blots loaded with 5, 2, 1, 0.5, 0.1 and 0.01 ng of a 1.5 kb N-ras insert on Hybond™-N+ nylon membrane. The 7th dot was a control of 5 ng carrier DNA. Results were obtained with CDP-*Star*™ Detection Reagent.

(A) (B) (C)

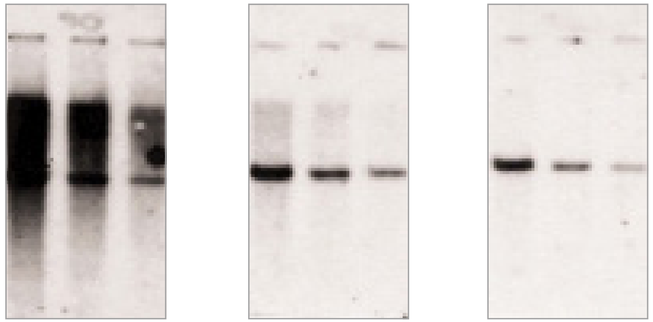

Fig 9. Control of stringency by wash temperature. Human genomic Southern blots (2, 1, and 0.5 µg loadings) hybridized with BCL2 at 5 ng/mL. Washed at (A) 50°C, (B) 55°C, and (C) 60°C. 0.2% blocking reagent included in the primary wash buffer.

Ordering information

Product	Pack size	Product code
AlkPhos Direct Labeling Module	For 2 500 cm² membrane	RPN3680
AlkPhos Direct Hybridization Buffer	For 5000 cm² membrane	RPN3688
CDP-*Star*™ Detection Reagent	For 2500 cm² membrane	RPN3682
ECF Substrate	For 2500 cm² membrane	RPN3685

Related products		Product code
ECL™ Direct Labeling and Detection System	To label 5 µg nucleic acid	RPN3000
	To label 10 µg nucleic acid	RPN3001
Hybond™-N+ membranes		See Table 4
Hyperfilm™ MP 18 × 24 cm	100 sheets	28906844
Hyperfilm™ MP 8 × 10"	100 sheets	28906846
Hyperfilm™ MP 5 × 7"	50 sheets	28906842
Hyperfilm™ MP 8 × 10"	50 sheets	28906845
Hyperfilm™ MP 18 × 24 cm	50 sheets	28906843
Hyperfilm™ MP Enveloped 18 × 24 cm	50 sheets	28906850

ECL™ Direct Labeling and Detection System

The ECL™ Direct Labeling and Detection System is based on the direct labeling of DNA or RNA probes with the enzyme horseradish peroxidase (HRP) in a simple chemical reaction. Labeling 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.

Protocol summary

Probe labeling

The labeling solution is added to the unlabeled, 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. Labeled probes can be stored in 50% glycerol for several months at -15°C to -30°C.

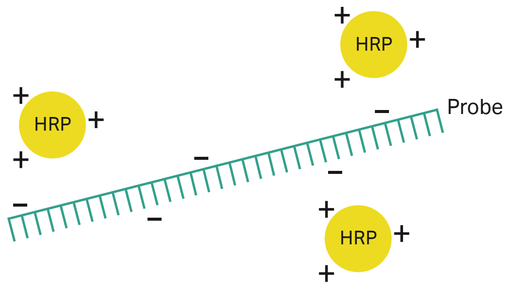

Fig 10. Probe labeling.

Hybridization

The labeled probe is hybridized to the blot using the specially formulated ECL™ Gold Hybridization 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.

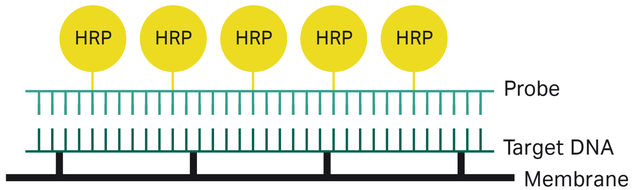

Fig 11. Hybridization.

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.

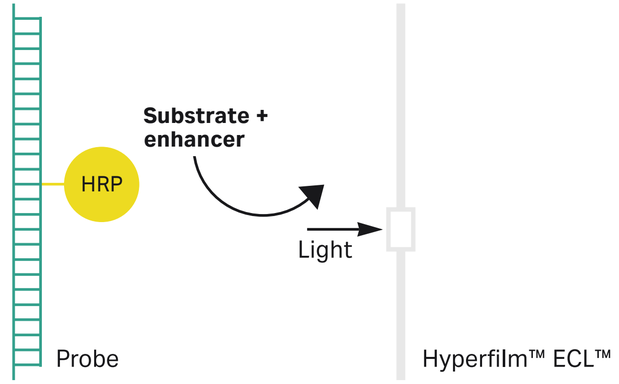

Fig 12. Detection.

Recommended applications

ECL™ Direct Labeling and Detection System 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.

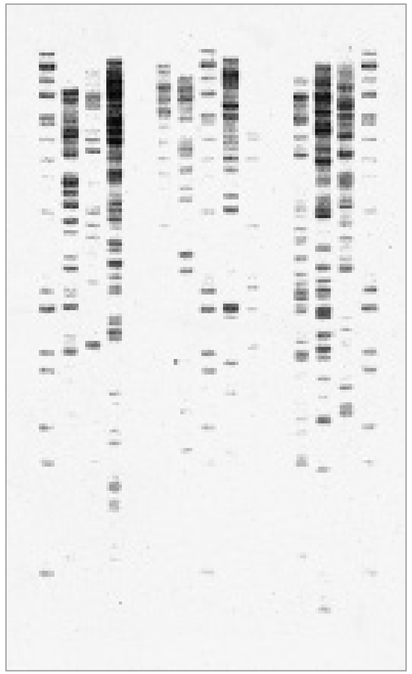

Fig 13. Random YAC DNA digested with Pvu*II* and hybridized with Alu*I* human DNA repetitive probe (exposure time of 30 minutes), using ECL™ Direct Labeling and Detection System.

Ease of use: Simple protocol coupled with high initial signal output provides a rapid non-radioactive system.

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

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

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

Convenience: Convenient, ready-to-use ECL™ Gold Hybridization Buffer formulated with an exclusive rate enhancer*.

Efficiency: DNA or RNA probes are labeled equally efficiently.

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

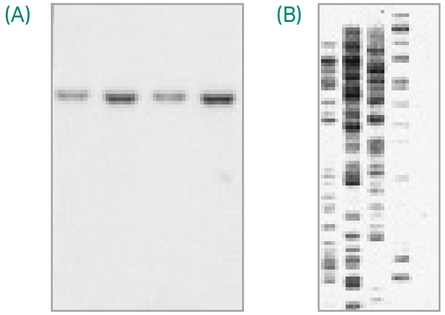

Fig 14. Comparison of 1st and 6th re-probing of Southern blotted human genomic DNA bound to Hybond™-N+ nylon membrane. Minimal reduction in signal intensity is observed. A) 6th re-probing, B) 1st probing.

Ordering information

Product	Pack size	Product code
ECL™ Direct Labeling and Detection System	To label 5 µg nucleic acid	RPN3000
	To label 10 µg nucleic acid	RPN3001
ECL™ Direct Detection Reagents	For 2000 cm^2 membrane	RPN3004
	For 4000 cm^2 membrane	RPN2105
ECL™ Gold Hybridization Buffer	For 4000 cm^2 membrane	RPN3006
ECL™ Direct Labeling System	To label 5 ng nucleic acid	RPN3005

Choosing a radioactive labeling system

Labeling with ^{32}P-labeled nucleotides remains a sensitive and robust technology.

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. A high-emission energy label will provide sensitive results with shorter exposure times, but resolution will be compromised.

Fig 15. Ready-To-Go™ pre-dispensed reagent beads.

In applications where resolution is most important, such as microsatellite analysis and sequencing, a lower energyemitting 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-labeled probes. Labeled 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 well-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.

Radiolabeled probes offer the following benefits:

- **Sensitivity**
  ^{32}P-labeled probes offer a high level of sensitivity, an excellent choice for detection where there is a very low abundance of target.
- **Prolonged emission**
  Radioactively labeled blots continue to emit signal for several weeks, enabling multiple exposures and long exposure times as required.
- **Reliability**
  Radioactive labeling 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 radiolabeled nucleotides into a probe can be controlled by the labeling conditions. Therefore probes can be produced with differing specific activities and incorporation levels can be measured accurately to determine exact probe concentrations.

Choice of labeling method

Random prime

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

Ready-To-Go™ DNA Labeling Beads, Amersham™ Rediprime II DNA Labeling System, Amersham™ Megaprime DNA Labeling System, 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 labeled 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 labeled 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.

RNA labeling

This technique produces radiolabeled 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.

End labeling

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

Table 3. Guide to the properties of radioactive labeling systems

Labeling system	Technology	Nucleotide	Amount of template	Labeling time	Probe specific activity (dpm/µg)	Recommended application
Amersham™ Rediprime II DNA Labeling System	Random-prime	dCTP only	25 ng	10 minutes	2×10^9	Membrane hybridization
Ready-To-Go™ DNA Labeling Beads	Random-prime	dCTP only	10 ng–1 µg	5 minutes	2×10^9	Membrane hybridization
Amersham™ Megaprime DNA Labeling System	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 labeling	SP6/T7 RNA polymerase	UTP	1 µg	1–2 hours	2×10^9	*In situ* hybridization
5′-end labeling	T4 polynucleotide kinase	dATP	10 pmol ends	1 hour	5×10^6	Membrane and *in situ* hybridization
3′-end labeling	Terminal deoxynucleotidyl transferase	Any dNTP	10 pmol ends	30–60 minutes	5×10^6	Membrane and *in situ* hybridization

Amersham™ Rediprime II DNA Labeling System

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

Amersham™ Rediprime II DNA Labeling System reaction mixes have been formulated using improved hyphenate 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, Amersham™ Rediprime II DNA Labeling Systemreactions can be set up and completed to produce a DNA probe ready for hybridization in less than 15 minutes.

Fig 16. Amersham™ Rediprime II DNA Labeling System with 30 pre-mixed labeling reactions for use with radiolabeled dCTP.

Recommended applications

Labeling 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 110 TBq/mmol, 3000 Ci/mmol.

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

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

Flexibility: DNA can be labeled in the presence of low melting point agarose or restriction enzyme buffers.

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

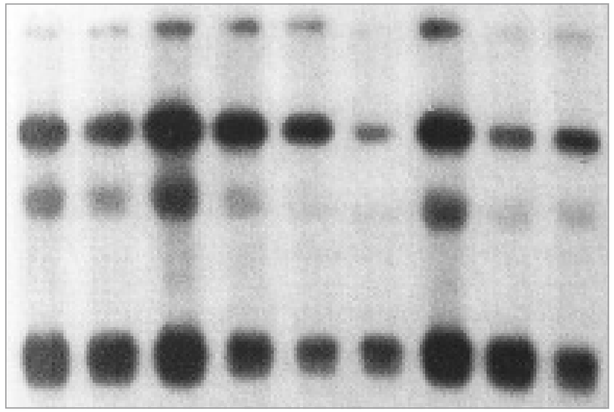

Fig 17. Northern blot probed with 3.8kb human EGFR cDNA fragments labeled with Amersham™ Rediprime II DNA Labeling System. Result kindly supplied by J M Loughlin, Zeneca Pharmaceuticals, UK.

Protocol summary

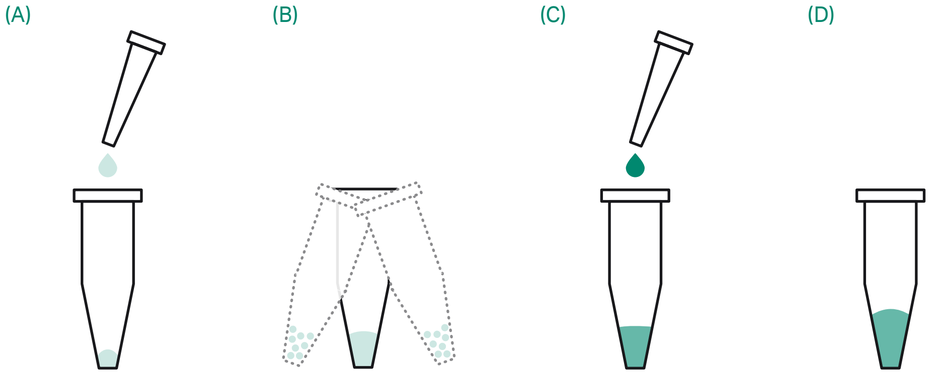

Fig 18. Schematic diagram of the Amersham™ Rediprime II DNA Labeling System protocol. (A) Add denatured template to a final volume of 45 µL, (B) flick tube and spin briefly, (C) add 5 mL Redivue [^{32}P]dCTP, pipette up and down and spin briefly and, (D) incubate for 10 minutes at 37°C.

Ordering information

Product	Pack size	Product code
Amersham™ Rediprime II DNA Labeling System	30 pre-mixed labeling reactions For use with radiolabeled dCTP	RPN1633

Related products		Product code
Rapid-hyb Buffer	For 1000 cm² membrane 125 mL	RPN1635
	For 4000 cm² membrane 500 mL	RPN1636
Hybond™ nylon membranes		See Table 4

Ready-To-Go™ RT-PCR Beads

Pre-dispensed, freeze-dried beads that include the reagents necessary for one-step reverse transcription-PCR with high sensitivity and reproducibility.

Ready-To-Go™ RT-PCR Beads are stable at room temperature and designed for performing single-tube one-step reverse transcription-PCR. Each room-temperature-stable bead contains M-MuLV Reverse Transcriptase, RNase Inhibitor, buffer, nucleotides, and Taq DNA Polymerase. The only additional reagents required are water, template RNA, and primers. The reagents are optimized for full-length cDNA synthesis to >7.5 kb and optimal sensitivity from PCR.

The Ready-To-Go™ Bead format significantly reduces the number of pipetting steps, thereby increasing reproducibility of the RT-PCR technique and minimizing risk of contamination and RNA degradation. Ready-To-Go™ RT-PCR Beads are provided in either thin walled 0.5 mL or 0.2 mL tubes compatible with most thermocyclers. The 0.2 mL tubes come assembled in a 96-well (8 × 12) plate format that allows individual strips of eight tubes to be easily removed. This flexibility allows use of either the entire 96-well plate, strips of eight or individual 0.2 ml tubes. Each package of Ready-To-Go™ RT-PCR Beads contains: RT-PCR beads, control reactions and pd(N)6 and oligo(dT) cDNA primers.

Convenience: Preformulated, predispensed, single-dose, ambient-temperature-stable beads ensure greater reproducibility between reactions, minimize pipetting steps, and reduce the potential for pipetting errors and contamination.

Compatibility: Optimized as a one-tube, one-step RT-PCR reaction for both cDNA synthesis and PCR.

Consistency: Each lot of RT-PCR Beads is function tested for its ability to generate highly specific PCR products to ensure lot-to-lot reproducibility in one-step reverse transcription-PCR.

Ordering information

Product	Pack size	Product code
Ready-To-Go™ RT-PCR Beads	0.2 mL hinged tube with cap	27925901
	0.2 mL tubes	27926701
	0.5 mL tubes	27926601

PuReTaq™ Ready-To-Go™ PCR Beads

Pre-formulated and pre-dispensed, freeze-dried PCR reaction mixes for robust and reproducible performance in PCR applications.

Ready-To-Go™ PCR Beads and reagents ensures reliable and robust performance in both end point and RT-PCR amplifications and ensures the lowest possible levels of contaminating prokaryotic and eukaryotic nucleic acids.

The only additional reagents required are water, primers, and template DNA. The beads are provided pre-dispensed into either 0.2 mL or 0.5 mL PCR tubes. The 0.2 mL tubes are also supplied in a 96-well (8 × 12) plate format that allows individual strips of eight tubes to be easily removed. This flexibility allows use of either the entire 96-well plate, strips of eight, or individual 0.2 mL tubes.

Convenience: Save time by simply adding template DNA solution and primers, then cycle. Long-term ambient-temperature stable at room temperature PCR reaction mixes means no freezer space is required and less energy consumption for shipping and storage.

Performance: High-quality PuReTaq™ DNA polymerase and high-purity reagents ensure robust performance and the lowest possible levels of contaminating DNA for more reproducible results, less risk of pipetting errors and contamination.

Ordering information

Product	Pack size	Product code
Ready-To-Go™ PCR Beads	0.2 mL hinged tube with cap, 96 reactions	27955901
	0.5 mL tubes, 100 reactions	27955801
	Multiwell plates, 5 × 96 reactions	27955702
	Multiwell plate, 96 reactions	27955701

Rapid-hyb Buffer

Rapid-hyb Buffer is a rate enhancing hybridization buffer for rapid hybridization of radiolabeled 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 and slot blots, and colony and plaque lifts.

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

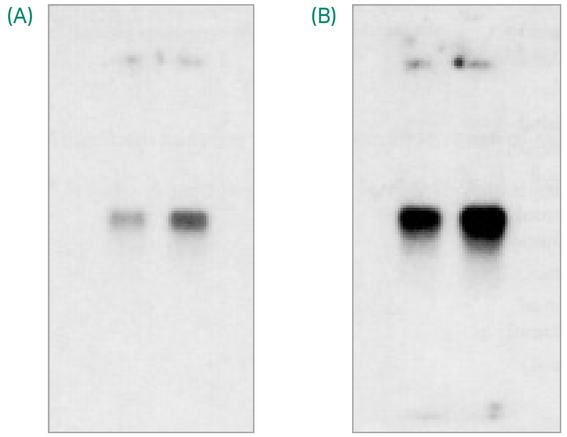

Fig 19. Northern blot analysis using (A) standard hybridization buffer and (B) Rapid-hyb Buffer. Northern blots of HeLa cell total RNA (0.5 µg loadings). Linearized pHSP70 probe labeled with (α-^{32}P)dCTP using Amersham™ Megaprime DNA Labeling System. Hybridizations were at 65°C for 1 hour using a probe concentration of 2 ng/mL. Exposure to Hyperfilm™ MP overnight.

Ordering information

Product	Pack size	Product code
Rapid-hyb Buffer	For 1000 cm² membrane 125 mL	RPN1635
	For 4000 cm² membrane 500 mL	RPN1636

Related product	Product code
Amersham™ Rediprime II DNA Labeling System	RPN1633

Hybond™ membranes for nucleic acid blotting

Hybond™ membranes are available to support nucleic acid blotting applications. Use Table 4 to select the most suitable membrane for you application.

Hybond™-XL membranes: This 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 membranes produce 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+ membranes: This is a positively charged nylon membrane which yields excellent sensitivity in both alkali blotting and conventional Southern blots. 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 membranes: This is a neutral nylon membrane capable of high sensitivity in DNA and RNA blotting. This strong supported membrane 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 membranes very well-suited for any standard radioactive Southern or northern blotting procedure, except for customers using low hybridization volumes where Hybond™-NX membrane is recommended.

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

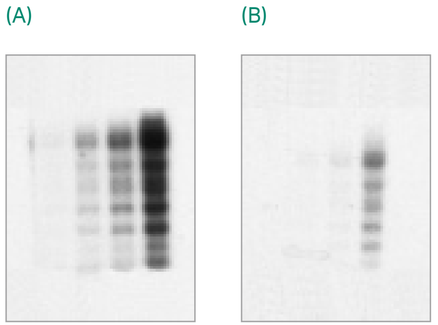

Fig 20. Human genomic Southern blot using (A) Hybond™-XL membrane and (B) leading competitor membrane. Human genomic DNA, digested with Hind III 2, 1, 0.5, 0.1 µg loadings. GAPDH random prime [^{32}P] labeled probe, 2 × 10^{6} cps/mL. Church hybridization buffer. Overnight exposure to Hyperfilm™ MP.

Table 4. Choosing the correct membrane

Recommended membrane			Southern blotting					Northern blotting				Dot and slot bots		Colony and plaque blots	
Product	**Format**	**Product code**	Radioactive detection	Non-radioactive detection	Alkali blotting	Low volume hybridizations	Rapid-hyb Buffer	Radioactive detection	Non-radioactive detection	Low volume hybridizations	Rapid-hyb Buffer	Radioactive detection	Non-radioactive detection	Radioactive detection	Non-radioactive detection
Hybond™-XL membrane	10 × (15 × 20 cm) sheets	RPN1520S	●		●		●	●			●	●		●	
	30 cm × 3 m roll	RPN303S	●		●		●	●			●	●		●	
	10 × (20 × 20 cm) sheets	RPN2020S	●		●		●	●			●	●		●	
	10 × (22.2 × 22.2 cm) sheets	RPN2222S	●		●		●	●			●	●		●	
	20 cm × 3 m roll	RPN203S	●		●		●	●			●	●		●	
Hybond™-N+ membrane	50 × 82 mm discs	RPN82B		●	●				●				●		●
	50 × (11.9 × 7.8 cm) sheets	RPN119B		●	●				●				●		●
	20 × (12 × 10 cm) sheets	RPN1210B		●	●				●				●		●
	10 × (15 × 20 cm) sheets	RPN1520B		●	●				●				●		●
	20 × (15 × 10 cm) sheets	RPN1510B		●	●				●				●		●
	30 cm × 3 m roll	RPN303B		●	●				●				●		●
	20 cm × 3 m roll	RPN203B		●	●				●				●		●
	10 × (20 × 20 cm) sheets	RPN2020B		●	●				●				●		●
	10 × (22.2 × 22.2 cm) sheets	RPN2222B		●	●				●				●		●
Hybond™-NX membrane	30 cm × 3 m roll	RPN303T				●				●			●		
	20 cm × 3 m roll	RPN203T				●				●			●		

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For local office contact information, visit cytiva.com/contact

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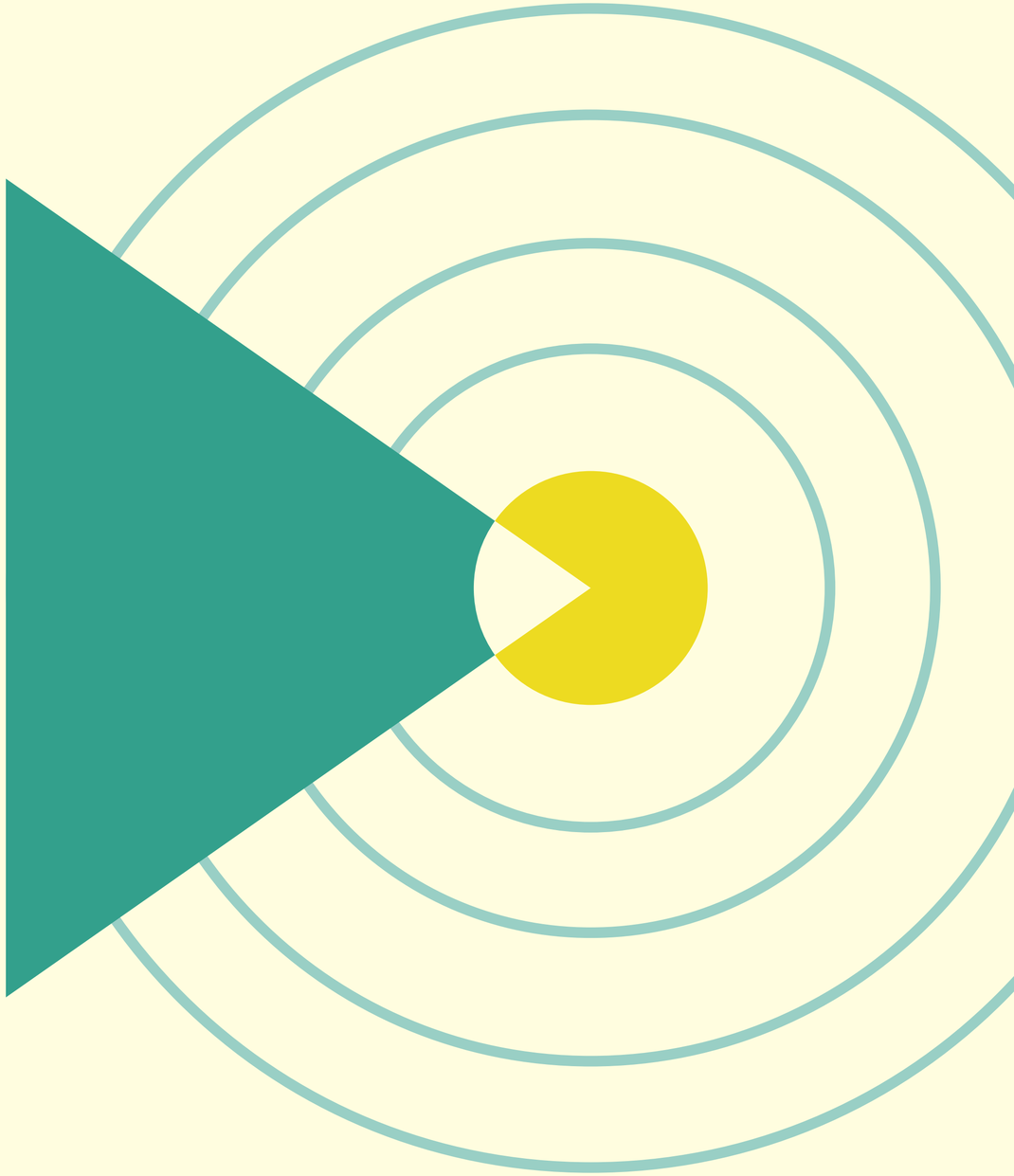

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