GE Healthcare

Amersham Rediprime II Random Prime Labelling System

Product Booklet

Codes: RPN1633
RPN1634

Page finder

1. Legal

http://www.gehealthcare.com/lifesciences

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: For use with radioactive material.
This product is to be used with radioactive material. Please follow the manufacturer's instructions relating to the handling, use, storage and disposal of such material.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.Wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at 2–25°C.

2.3. Expiry

The kit components are stable for up to 6 months when stored under the recommended conditions.

3. Components of the system

Rediprime II Random Prime Labelling System

Component	RPN 1633	RPN 1634
Labelling reaction Buffered solution of dATP, dGTP, dTTP, exonuclease free Klenow enzyme and random primers in a dried, stabilised form	30 reactions	60 reactions
Control DNA 300 ng of lambda *Hin*d III DNA in a dried, stabilised form	1 tube	2 tubes
Protocol summary card		

4. Description

Feinberg and Vogelstein[1,2] introduced the use of random sequence hexanucleotides to prime DNA synthesis on denatured template DNA at numerous sites along its length. The primer-template complex is a substrate for the Klenow fragment of DNA polymerase I. By replacing a non-radioactive nucleotide with the radiolabelled equivalent in the reaction mixture, newly synthesised DNA is made radioactive.

Very small amounts of input DNA can be labelled, enabling very high specific activity probes to be produced with relatively small quantities of added nucleotides. These radioactive labelled fragments can then be used as sensitive hybridisation probes for a wide range of filter based applications [3-6].

Traditional protocols for the random primer labelling of DNA required reaction times of at least 30 minutes. More recent procedures, such as that used in GE Healthcare popular Megaprime™ DNA labelling system, allow the labelling of template DNA to the same specific activity but at a greatly accelerated rate. This rapid labelling at 37°C is achieved by the use of primers at high concentration giving more efficient priming from the template.

Rediprime™ random prime labelling systems from GE Healthcare have been developed for extra convenience and performance. The systems provide individually dispensed reaction mixes which are dried in the presence of a stabiliser and a dye to make labelling probes easier for the user. There is no requirement to store the systems in a freezer, they can be stored in the fridge or on the laboratory bench ready for use. Rediprime reaction mixes have been formulated using an improved exonuclease-free Klenow to give probes with specific activity of 1.9×10^9 dpm/µg or greater after 10 minutes incubation at 37°C with the majority of DNA substrates. When used with Redivue™ [^{32}P]dCTP, Rediprime reactions can be set up and completed to produce a DNA probe ready for hybridisation in 20 minutes.

5. Critical parameters

To get high labelling efficiencies, there are two critical factors:

- TE buffer must be used as a diluent for the DNA template.
- The DNA template must be boiled in a total volume of 45 µl.
- Solutions which are too dilute to be used directly should be concentrated by ethanol precipitation. Redissolution in a volume ≤45 µl of 10 mM Tris/HCl pH 8.0, 1 mM EDTA is recommended.

6. Additional equipment and reagents required

- Pipettes or pipetting equipment for 5, 50 and 100 µl
- TE buffer
- Boiling water bath
- 37°C water bath or heating block
- 0.2 M EDTA
- Refrigerator
- Freezer

7. Rediprime II Random Prime Labelling System protocol

It is recommended that the protocol is read thoroughly before using the system.

Rediprime allows DNA from a variety of sources to be labelled to high specific activity using [^{32}P]dCTP. The system has been designed for use with Redivue [^{32}P]dCTP with a specific activity of 3000 Ci/mmol.

Each reaction tube can label up to 25 ng of DNA and, after incubation for 10 minutes at 37°C, probes with a specific activity of 1.9×10^9 dpm/µg or greater can be produced.

DNA prepared by standard minilysate methods may be used. DNA in restriction enzyme buffers may be added directly to the reaction, and the reaction can also be performed with DNA in low melting point agarose gel slices (see page 11).

Protocol	Notes
1. Dilute the DNA to be labelled to a concentration of 2.5–25 ng in 45 µl of 10 mM Tris HCl pH8.0, 1 mM EDTA. (TE buffer)	**1.** To get high labelling efficiencies, it is important that TE buffer is used and that the final volume used to reconstitute the mix equals 45 µl.
2. Denature the DNA sample by heating to 95–100°C for 5 minutes in a boiling water bath.	
3. Snap cool the DNA by placing on ice for 5 minutes after denaturation.	

Protocol	Notes
4. Centrifuge briefly to bring the contents to the bottom of the tube.	
5. Add the denatured DNA to the reaction tube.	**5.** Do not mix at this stage.
6. Add 5 µl of Redivue [^{32}P] dCTP and mix by pipetting up and down about 12 times, moving the pipette tip around in the solution.	**6.** The denatured DNA solution will change colour to purple when the blue pellet has been completely dissolved and mixed with the Redivue.
7. Incubate at 37°C for 10 minutes.	**7.** If desired, the labelling reaction can be allowed to proceed at room temperature, or may also be left to proceed overnight. Probes of high specific activity can be generated in 20–60 minutes at this temperature (see figure 1).
8. Stop the reaction by adding 5 µl of 0.2 M EDTA. For use in hybridisation, denature the labelled DNA by heating to 95–100°C for 5 minutes, then snap cool on ice for 5 minutes.	
9. Centrifuge the tube briefly and mix the contents of the tube well.	

Protocol	Notes
10. We recommend that if 25 ng of template was used in the labelling reaction, then 14 µl of this labelled probe is used per 5 ml of hybridisation buffer.	**10.** Recommended probe concentration based on template quantity is 1.4 ng/ml. This is likely to give an actual probe concentration of 2.8 ng/ml due to the amount of probe synthesised during the reaction.

8. Additional Information

8.1. Quality control

Rediprime random prime labelling systems are tested by our quality control group to ensure an incorporation rate greater than 55% after 10 minutes at 37°C using 50 µCi Redivue [^{32}P]dCTP and to detect a band in a human genomic Southern blot equivalent to 0.5 pg following a 2 hour hybridisation with overnight film exposure.

8.2. Using the control DNA

The performance of Rediprime systems can be checked by using the control DNA supplied. The vial contains 300 ng of lambda DNA which should be dissolved in TE buffer before use. Once reconstituted 5 µl of control DNA contains 25 ng. It may be stored at 2–8°C for up to 1 month, or for longer periods at -15°C to -30°C.

Protocol

1. Reconstitute the DNA by adding 60 µl of TE buffer and flick the tube until the DNA has dissolved.

2. Spin briefly and proceed with steps 1–8 of the Rediprime protocol.

8.3. Use of alternative reaction conditions

Labelling at room temperature

If required, labelling reactions can be carried out at room temperature. Typical results for labelling at this temperature are shown in figure 2. Room temperature should be used if reactions are to be left to incubate overnight.

Labelling of DNA fragments in low melting point agarose[7]

1. Cut out the DNA band from an ethidium bromide stained agarose gel and trim off excess agarose.

2. Weigh the agarose plug and add 3 ml of water for each gram of gel.

3. Place in a boiling water bath for 5–10 minutes to melt the gel and to denature the DNA. Store the DNA solution at 37°C until required.
4. Remove an aliquot and add 10 mM Tris HCl pH 8.0, 1 mM EDTA. (TE buffer) to give a final volume of 45 µl containing 2.5–25 ng DNA.
5. Proceed from step 4 of the Rediprime protocol, extending the labelling reaction time to 15–30 minutes at 37°C.

Labelling using less [^{32}P]dCTP

For maximum probe specific activity (1.9 × 10^9 dpm/µg or greater) and sensitivity in hybridisations the recommended 50 µCi of [^{32}P]dCTP should be used in all reactions. In certain cases, where a lower specific activity probe may be acceptable, the amount of [^{32}P]dCTP may be reduced. For example, using 20 µCi [^{32}P]dCTP will typically yield probes of specific activity up to 1x 10^9 dpm/µg.

8.4. Monitoring the reaction and calculating the specific activity of labelled DNA

- It is possible to monitor the reaction using DE81 paper[8].
- It is also possible to monitor the reaction using precipitation with TCA (trichloroacetic acid)[9].
- The specific activity of labelled DNA can be calculated using the following formulae. First calculate the amount of DNA (template + probe) existing at the end of the reaction;

$$\text{Mass of DNA (ng)} = \frac{[\mu\text{Ci added}][13.2][\%\text{incorporation}]}{\text{Specific activity of } [^{32}\text{P}]\text{dCTP}} + \text{starting template (ng)}$$

For example, if you have labelled 25 ng of DNA using 50 µCi of Redivue[^{32}P] dCTP at a specific activity of 3000 Ci/mmol, and your incorporation is 70%;

$$\text{Mass of DNA} = \frac{[50\ \mu\text{Ci}][13.2][70\%]}{3000} + 25 = \mathbf{40.4\ ng}$$

Next, calculate the amount of radioactivity incorporated during the reaction in dpm;

$$=[50\ \mu Ci][2.2 \times 10^4][70\%] = \mathbf{7.7x10^7\ dpm}$$

The specific activity of the labelled DNA can now be calculated as:

$$\frac{[\text{dpm incorporated}][10^3]}{\text{DNA mass}} \quad \text{ie} \quad \frac{[7.7x10^7\ \text{dpm}][10^3]}{40.4\ \text{ng}}$$

= **$1.9x10^9$ dpm/µg**

8.5. Removal of unincorporated nucleotides

Removal of unincorporated nucleotides is sometimes desirable to reduce background during hybridisation, particularly if incorporation is less than 50%.

It is also considered important to remove these free nucleotides if the probe is being kept for several days before being used. However, if GE Healthcare's new Rapid-hyb™ buffer is used, purification is not required unless the probe is to be stored for more than 24 hours before use. Probes can be purified by Sephadex™ chromatography or selective precipitation[9,10].

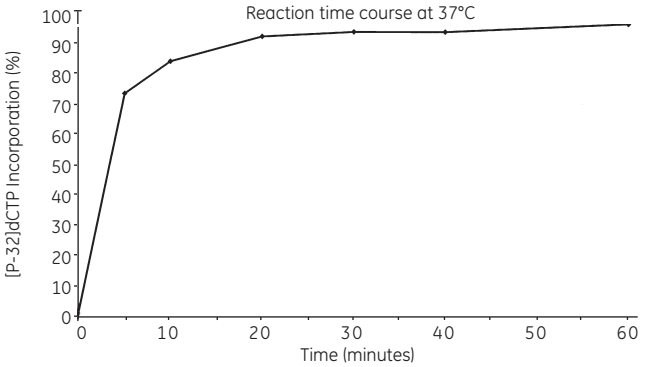

Fig 1. Time course of incorporation of μ-^{32}P]dCTP (17 pmols) in a Rediprime reaction at 37°C using the control DNA supplied with the system.

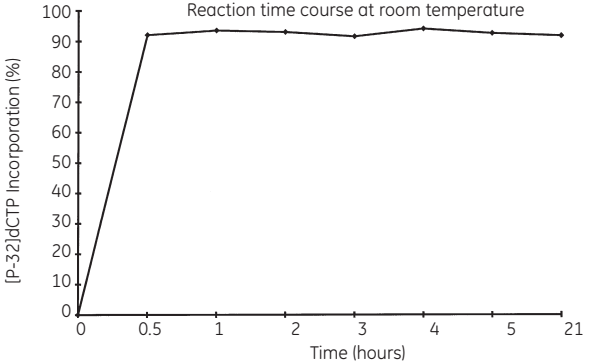

Fig 2. Time course of incorporation of [μ-^{32}P]dCTP (17 pmols) in a Rediprime reaction at room temperature using the control DNA supplied with the system.

9. Related products

^{32}P Nucleotides

Redivue stabilised [α-^{32}P]dCTP	AA 0005
[α-^{32}P]dCTP	PB 10205

Labelling systems

Megaprime DNA labelling system for use with any radiolabelled nucleotide	RPN 1604/5
Megaprime DNA labelling system for use with radiolabelled dCTP	RPN 1606/7
Nick translation kit	N 5000/5500
3'-end labelling kit	N 4020
5'-end labelling kit	RPN 1509
RNA labelling system	RPN 3100

Hybridisation buffers

Rapid-hyb buffer	RPN 1635/6
Hybridisation buffer tablets	RPN 131

Hybridisation products

Hybond™ range of nylon and nitrocellulose blotting membranes	
UV Crosslinker(220/240V 50Hz)	RPN 2500
UV Crosslinker(110/115V 60Hz)	RPN 2501
Hybridisation oven/shaker (220/240V 50Hz)	RPN 2510
Hybridisation oven/shaker (110/115V 60Hz)	RPN 2511

Autoradiography products
Hyperfilm™ high performance autoradiography films.
Hypercassette™ and Hyperscreen™ available from stock.

Safety products
Radiation safety products for safe handling and storage of ^{32}P.

Agarose
SepRate™ highly purified agarose for DNA fragments of different sizes and uses.

See our current catalogues or contact your local GE Healthcare office for further details.

10. References

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4. THOMAS, P.S., *Proc. Nat. Acad. Sci.*, USA., **77**, pp.5201-5205, 1980.
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RPN1633PL Rev B 2006

Amersham
Rediprime II Random Prime Labelling System
Product protocol card RPN1633PC

Add denatured DNA template in a final volume of 45 µl.

Add 5µl of Redivue™ [^{32}P] dCTP.

Pipette up and down to mix minutes at 37°C.

Incubate for 10 minutes at 37°C.

Caution: For use with radioactive material.

This product is to be used with radioactive material. Please follow the manufacturer's instructions relating to the handling, use, storage and disposal of such material.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Storage and stability

Store Rediprime™II random prime labelling system between 2–25°C. The system is stable for up to 6 months when stored under these recommended conditions.

Using the control DNA

The performance of Rediprime II can be checked by using the control DNA supplied.
The vial contains 300 ng of lambda HindIII DNA which should be dissolved in 60 µl TE buffer before use by pipetting up and down. Once reconstituted 5 µl of control DNA contains 25 ng template.

Ordering information	**Code**
Rediprime II DNA labelling system	RPN 1633
Redivue™ stabilised [α-^{32}P]dCTP	AA 0005

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RPN1633PC Rev B 2006