GE Healthcare

Amersham Ready-To-Go DNA Labelling Beads (-dCTP)

Product Booklet

Code: 27-9240-01

Page finder

1. Legal

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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.

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.

Warning: Several of the reagents recommended in these instructions (such as trichloroacetic acid and ethidium bromide) are toxic or corrosive and should be handled with care.

2.2. Storage

Store at ambient room temperature in the airtight foil pouch with the desiccant. Once opened, completely reseal the pouch, fold the sealed edge over several times and seal with a clip. Store unopened and resealed pouches in a desiccator to maximize product lifetime. Store reconstituted Control DNA at 4°C.

3. Components of the kit

Reaction Mix	Room-temperature-stable bead containing buffer, dATP, dGTP, dTTP, FPLCpure™ Klenow Fragment (7–12 units) and random oligodeoxyribonucleotides, primarily 9-mers. Twenty tubes supplied.
Control DNA	Room-temperature-stable bead containing restriction fragments from α λ DNA-Hind III digest (stable at room temperature until reconstituted, then to be stored at 4°C). See page 10 for information on reconstitution.

For additional reagents required, see Materials not Supplied.

4. Quality control

Each batch of Ready-To-Go™ DNA Labelling Beads (-dCTP) is tested for its ability to label 25 ng of the Control DNA with ^{32}P to a specific activity of at least 1×10^9 dpm/µg within 15 minutes.

5. Materials not supplied

Reagents

- **Labelled dCTP** - Radio-actively labelled dCTP is available in a wide range of specific activities. For [α-^{32}P]dCTP, 3000 Ci/mmol is recommended (GE Healthcare PB 10205 or AA 0005).
- **TE buffer** - 10 mM Tris-HCl (pH 8), 1 mM EDTA.
- **0.2 M EDTA (pH 8)**
- **10 mM EDTA (pH 8)**
- **Trichloroacetic acid (TCA)** - 10% (v/v)
- **Carrier DNA** - Sonicated Calf Thymus DNA (27-4563-01, 02) or Sonicated Salmon Sperm DNA (27-4565-01, -02) at a concentration of 1 mg/ml.

6. Introduction

"Oligolabelling" was developed as a method for labelling DNA restriction fragments to high specific activity for use as hybridization probes (1, 2). The DNA to be labelled is first denatured and then mixed with oligodeoxyribonucleotides of random sequence. These "random oligomers" anneal to random sites on the DNA and then serve as primers for DNA synthesis by a DNA polymerase. With labelled nucleotide(s) present during this synthesis, highly labelled DNA is generated.

Ready-To-Go DNA Labelling Beads are manufactured by a proprietary technology licensed to GE Healthcare. Each kit contains pre-dispensed reaction mixes which are treated by a special process which turns each one into a bead, stable at ambient temperatures. Each Reaction Mix is quickly reconstituted when mixed with a denatured DNA solution. Thus, with Ready-To-Go DNA Labelling Beads, preparation and setup are convenient: The Reaction Mix bead is simply reconstituted with a solution of denatured template, label is added and the tube is placed at 37°C for 5–15 minutes. The labelled probe is then available for immediate use.

Each reaction is capable of labelling between 10 ng and 1 µg of linearized DNA. Using 25–50 ng of DNA and [α-^{32}P]dCTP at 3 000 Ci/mmol, a specific activity of > 1 x 10^9 dpm/µg can be achieved, in as little as 5–15 minutes.

Standard protocol

The procedure below is a starting point to label 25–50 ng of DNA to > 1 x 10^9 dpm/µg. Please refer to pages 8–18 for information to optimize the labelling reaction if needed.

The linearized DNA should be dissolved in TE buffer, at a concentration such that 25–50 ng can be added to the reaction in a volume of no more than 45 µl.

1. Check that the bead is visible in the bottom of the tube of Reaction Mix. If not, tap against a hard surface to bring the bead to the bottom of the tube.
2. Denature the DNA by heating for 2–3 minutes at 95–100°C. Immediately place on ice for 2 minutes, then centrifuge briefly.
3. Add the following to the tube containing the Reaction Mix bead:

Denatured DNA (25–50 ng)	≤ 45 µl
[α-^{32}P]dCTP (3 000 Ci/mmol)	5 µl (50 µCi)
Distilled water	to total of 50 µl

4. Mix by gently pipetting up and down several times or gently vortexing. Bubbles may be removed by a pulse centrifugation.
5. Incubate at 37°C for 5–15 minutes. (Difficult template may require up to 30 minutes.)

In general, the labelled DNA may be used as a hybridization probe without stopping the reaction.

7. Protocols

7.1. Design of labelling protocols

Factors to be considered in designing a labelling protocol include preparation and quantity of template, choice and quantity of labelled nucleotide and reaction time.

Template preparation

Oligolabelling may be applied to DNA either in solution or in low-melting-point agarose. Plasmid DNA to be labelled may be prepared by either of the popular "miniprep" methods (3). GFX™ *Micro* Plasmid Prep Kit (GE Healthcare 27-9601-02) offers a particularly convenient method for preparing plasmid DNA suitable for labelling.

If the entire plasmid is to be labelled, contaminating RNA should first be removed; it will otherwise anneal with the random oligomers, interfering with the desired reaction. Plasmid DNA isolated using GFX *Micro* Plasmid Prep Kit (27-9601-02) is virtually free of RNA and may be used without any further purification. Alternatively, contaminating RNA can be conveniently removed from plasmid samples using a MicroSpin™ S-300 HR Column (27-5130-01). The plasmid should also be linearized with a restriction enzyme to ensure effective denaturation prior to labelling.

Specific restriction fragments which are to be labelled should be separated by electrophoresis in gels of low-melting-point agarose and recovered as described in Protocol 7.1. Alternatively, fragments can be eluted from agarose gels, precipitated, redissolved, and labelled in solution.

Control DNA

The Control DNA (restriction fragments from a digest of λ DNA with *Hind* III) must be dissolved in TE buffer before use. The tube contains 125 ng of DNA. We recommend adding 225 µl of TE buffer, to obtain

a concentration of 0.56 ng/µl. Forty-five microliters would then be used in a control reaction.

Quantity of DNA to be labelled

In our laboratories, oligolabelling has been performed successfully on quantities of DNA as large as 1 µg. For radioactive labelling, however, the advantages of the procedure are most apparent for quantities of DNA up to about 100 ng; for larger quantities, the amount of labelled nucleotide required to obtain DNA of very high specific activity becomes expensive.

Choice and quantity of labelled nucleotide

Ready-To-Go DNA Labelling Beads (-dCTP) may be used with various forms of dCTP/analogues, labelled with ^{32}P, ^{33}P, ^{35}S, or biotin. The choice of labelled dCTP and quantity to use will depend on the specific activity required of the labelled DNA, which in turn will depend on the use intended for this DNA. The following *approximation* may be used as a general *guideline* to determine how much labelled nucleotide to use to obtain DNA of a given specific activity (SA):

$$SA = (\mu Ci/ng)(10^9)$$

where SA = *Specific activity of DNA (dpm/µg);*
μCi = *Quantity of input label (in µCi);*
ng = *Quantity of input DNA (in ng).*

In practice, the value for SA yielded by this equation will be reasonably accurate when using dCTP with a specific activity ≥ 3 000 Ci/mmol, but too high when using dCTP of lower specific activity (because in the latter case the *percentage* of the labelled nucleotide incorporated will tend to be lower, reflecting the fact that the same number of µCi represents more moles of dCTP). In general, 1 µCi of labelled nucleotide per 0.5–1 ng of input DNA will yield probes labelled to approximately 1×10^9 dpm/µg. For biotin-labelled

dCTP (bio-dCTP), we recommend the use of 5.0 nmol of nucleotide for labelling up to 50 ng of DNA.

Length of probe obtained

Using the Standard Protocol described on pages 8 and 9 to label λ DNA digested with *Hind* III, the probes obtained will cover the full range of fragment sizes, with a median size range of 200–1 000 nucleotides.

Probes labelled to high specific activity with ^{32}P undergo significant radiolytic decomposition within a few days of synthesis (4). In our hands, however, such probes may be used in blot hybridizations for up to 10 days with little loss of sensitivity.

Time course of reaction

The time course for the reaction is also influenced by the choice and quantity of labelled dCTP. The following generalities apply: (i) the higher the specific activity of the labelled nucleotide and the smaller the quantity of DNA being labelled, the shorter will be the incubation time required for incorporation of label to reach its maximum; and (ii) reaction times may need to be extended for DNA fragments in low-melting-point agarose.

Typical results are shown in Figure 1 (page 13). In this experiment, 25 ng of Control DNA was labelled with 50 µCi of [α-^{32}P]dCTP (3 000 Ci/mmol). Under these conditions, a specific activity of $\geq 1 \times 10^9$ dpm/µg may be rapidly achieved.

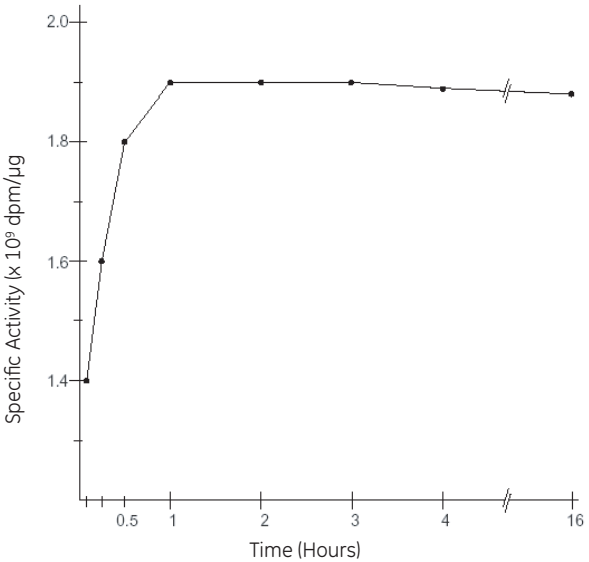

Figure 1. Time course of [α-^{32}P]dCTP incorporation during labelling

7.2. Preparation of template

DNA in solution

1. The linearized DNA should be dissolved in TE buffer (Materials Not Supplied, page 7). If necessary, adjust its concentration so that the amount which is to be labelled can be pipetted in ≤ 45 µl. The specific activity observed will be proportional to the amount of DNA to be labelled.

Note: The maximum volume of DNA solution which can be added to the labelling reaction will depend on the volume of labelled nucleotide required (adjusted for decay); the combined volumes of reconstituted Reaction Mix, DNA and labelled nucleotide must not exceed 50 µl.

2. Check that the bead is visible in the bottom of the tube of Reaction Mix. If not, tap against a hard surface to bring the pellet to the bottom of the tube.

3. Denature the DNA by heating for 2–3 minutes at 95–100°C. Immediately place the DNA on ice for 2 minutes, then centrifuge the tube briefly. The denatured DNA may be labelled immediately, or stored for up to 2 weeks at -20°C.

4. Proceed with the labelling reaction, Protocol 7.3., page 15.

DNA Fragments in low-melting-point agarose

5. Digest the DNA of interest with the appropriate restriction enzyme(s). Prepare a gel of low-melting-point agarose containing 0.05 µg/ml of ethidium bromide. Load and run a sufficient amount of the digested DNA so that the band of interest will contain at least 250 ng of DNA.

6. Cut out the desired band from the gel with a minimum of excess agarose. Place the agarose slice into a pre-weighed microcentrifuge tube and weigh the slice.

7. Add 3 ml of distilled water per gram of gel slice.

8. Heat the tube at 65–75°C for 2 minutes to melt the agarose.

9. Mix the sample gently to make a homogeneous solution. Store any material not to be used immediately at -20°C. When aliquots are subsequently required for labelling, melt the sample by heating at 65–75°C for 2 minutes.

Up to 45 µl of this material may be utilized in the labelling reaction. Prepare the diluted "DNA-agarose" for labelling as follows:

10. Heat in a water bath at 95–100°C for 7 minutes.

11. Transfer the solution to a 37°C water bath, and incubate for 10 minutes.

12. Remove the tube containing the DNA-agarose mixture from the water bath and centrifuge it briefly. Allow to cool to room temperature for 1–2 minutes before use.

13. Proceed with the labelling reaction.

7.3. Labelling reaction

This procedure may be used for labelling between 10 ng and 1 µg of DNA. See also pages 10–12.

1. Add the following to the microcentrifuge tube containing the Reaction Mix Bead:

Denatured DNA	≤ 45 µl*
[α-^{32}P]dCTP (3 000 Ci/mmol)	5 µl* (50 µCi)
Distilled water	to total of 50 µl

2. Mix by gently pipetting up and down several times or by gentle vortexing. If bubbles appear they may be removed by a pulse centrifugation.

3. Incubate for an appropriate period at 37°C. The following incubation times are recommended, although the reaction can be

allowed to proceed at either room temperature or 37°C for up to 24 hours:

For [^{32}P]dCTP	: 5–15 minutes**
For [^{33}P]dCTP	: 1–2 hours
For [^{35}S]dCTPαS	: 4 hours
For biotin-dCTP (0.1 mM final)	: 1 hour
For DNA from agarose slices	: 1–5 hours for ^{32}P

* The combined volumes of the reconstituted Reaction Mix, de-natured DNA and labelled nucleotide must not exceed 50 µl.

** Most DNA will be labelled to a high specific activity within 5–15 minutes. However, difficult templates may require incubation times of up to 30 minutes.

In general, the labelled DNA may be used as a hybridization probe without stopping the reaction. However, if you choose to stop the reaction, refer to Protocol 7.4. Protocol 7.4. also gives the protocol for removing unincorporated nucleotides prior to hybridization.

If desired, the progress of the reaction may be monitored as described in Protocol 7.3.

7.4. Monitoring progress of reaction

1. For labelling reactions which contain up to 50 µCi of dCTP, remove a 5 µl sample; for reactions containing more than 50 µCi, remove a 1 µl sample.

2. Dilute the sample taken from the reaction into 10 mM EDTA (pH 8) or water so that the final volume is 100 µl.

To determine "**total counts**" (T):

3. Spot 5 µl of the diluted sample onto the center of a glass-fiber filter disc and allow it to dry.

To determine "**acid-precipitable counts**" (P):

4. Transfer a second 5 µl aliquot of the diluted sample to a 1.5 ml microcentrifuge tube containing 25 µl of Carrier DNA (see "Materials Not Supplied").

5. Add 1 ml of ice-cold 10% (v/v) TCA solution. Leave on ice for 15 minutes to allow precipitation.

6. Collect the precipitated DNA onto a glass-fiber filter disc by vacuum filtration. Wash the filter with 10 ml of ice-cold TCA solution, then with ethanol, and allow it to dry.

Determine the amount of radioactivity on each filter using appropriate liquid scintillation counting techniques. Calculate the fraction of input radioactivity which has been incorporated (F) by dividing acid-precipitable counts by total counts; F=P/T.

The specific activity (SA) of the labelled DNA can now be determined as follows:

$$SA = \frac{(\mu Ci)(2.2 \times 10^9)(F)}{D_i + [(1.3 \times 10^3)(F)(\mu Ci/S)]}$$

where SA = Specific activity of DNA (dpm/mg);

mCi = mCi of dCTP in reaction;

F = Fraction of input label incorporated into DNA (=P/T);

D_i = Mass of input DNA template (in ng);

S = Specific activity of dCTP (in Ci/mmol = mCi/nmol).

Note: The numerator of this equation is derived by multiplying three factors: the total dpm in the reaction [(µCi)(2.2 x 10^6)]; the fraction of these which were incorporated (F); and a factor to convert the final value for SA from dpm/ng to dpm/µg (10^3).

Note: The denominator represents the total mass of DNA (in ng) at the end of the reaction, equal to the starting mass (D_i) plus the mass (in ng) synthesized during the reaction. The latter is calculated from the number of nanomoles of dCMP incorporated [(F)(µCi/S)],

multiplied by four times the average molecular weight of the four dNMPs [(4)(325) = 1.3 x 10^3].

7.5. Preparation for hybridization

In general, it is not necessary to stop the reaction. Removing unincorporated nucleotides prior to hybridization, while also not necessary, is recommended. If you choose to perform either of these steps, proceed as follows:

1. Stop the reaction by adding 5 µl of 0.2 M EDTA (pH 8).

2. Remove unincorporated nucleotides using a ProbeQuant™ G-50 Micro Column (27-5335-01). Alternatively, probes may be purified by gel filtration on Sephadex™ G-50 DNA Grade (17-0573-01, -02), using a 2 cm x 1 cm bed (height x diameter).

To prepare labelled DNA for hybridization:

3. Denature the DNA by heating at 95–100°C for 2 minutes. Cool immediately on ice.

Note: Denatured DNA may be stored at -20°C for up to 1 week.

8. References

1. Feinberg, A. P. and Vogelstein, B., *Anal. Biochem.* **132**, 6 (1983).

2. Feinberg, A. P. and Vogelstein, B., *Anal. Biochem.* **137**, 266 (1984).

3. Sambrook, J., Fritsch, E. F., and Maniatis, T., *Molecular Cloning: A laboratory manual,* Cold Spring Harbor Laboratory (second edition, 1989).

4. Hodgson, C.P., *et al, BioTechniques* **6**, 208 (1988).

9. Related products

Product	Pack size	Product number
Redivue™ [α-^{32}P]dCTP, triethylammonium salt	250 µCi–1 mCi	AA 0005
[α-^{32}P]dCTP, triethylammonium salt	250 µCi–1 mCi	PB 10205
GFX™ MicroPlasmid Prep Kit	250 purifications	27-9601-02
Microspin S-300 HR Columns	50	27-5130-01
ProbeQuant™ G-50 Micro Columns	50	25-5335-01

Please contact your local GE Healthcare representative for information on other DNA labelling and detection products, including hybridization membranes and films

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