**ALL-IN-ONE™**
**RANDOM PRIME DNA LABELING MIX (-dATP)**

# ProductInformation

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**TECHNICAL BULLETIN**

## Product Description

Sigma's All-In-One™ Random Prime DNA Labeling Mix is based on the method developed by Feinberg and Vogelstein.[1,2] The sample DNA is denatured and allowed to anneal with random 9-mers, which serve as primers for the Klenow fragment of DNA Polymerase I. Deoxyadenosine triphosphate (dATP) modified with $^{32}$P-, digoxin, fluorescein, or biotin, is incorporated into the newly synthesized DNA strand. This method allows the labeling of very small quantities of DNA and of DNA fragments that have been separated in low melting point agarose to high specific activities. After labeling, the DNA may be used as a probe in various hybridization applications.

Each All-In-One random prime reaction tube is ready for use after reconstitution with denatured sample DNA and the label of choice. Labeled nucleotide is not included and must be obtained separately.

## Reagents Provided

Each tube is sufficient for labeling 10 ng-1 µg of template DNA

- **Random Prime Reaction Tubes,**
  Product No. R7022 (1.5 ml tubes) or
  Product No. R9522 (0.2 ml tubes)
  Each reaction tube contains dCTP, dGTP, TTP, random 9-mers, and Klenow fragment of DNA polymerase I (15 units) lyophilized with reaction buffer
- **Control DNA,** Product No.D7684 1 vial
  λ Hind III, 125 ng/vial

## Reagents That May be Required But Not Provided

(Sigma product numbers are given where appropriate)

- 0.5 M molecular biology grade EDTA, Product No. E7889
- 100% trichloroacetic acid (TCA), Product No. 490-10
- α-$^{32}$P, biotin, digoxin, or FITC labeled dATP
- Glass fiber filter, >2.3 µm retention, or equivalent, e.g. Product No. F5019
- Molecular biology water, Product No. W4502
- 100 X TE Buffer (1 M Tris-HCl, pH 8, 100 mM EDTA), Product No. T9285
- Disodium pyrophosphate, Product No. P8135
- tRNA, Product No. R8508
- Vacuum manifold, Product No. M2536

## Precautions

Sigma's All-In-One Random Prime DNA Labeling Mix is for laboratory use only. Not for drug, household or other uses. If the sample DNA is radioactively labeled, standard procedures for safely handling radioactive materials should be followed.

## Storage

Store all kit components desiccated at room temperature. After reconstitution, store the control DNA at 2-8°C.

**Reagent Preparation**

1. Dilute 100X TE buffer concentrate 1:100 with molecular biology grade water to prepare 1X TE buffer.

2. Add 225 µl of 1X TE buffer to the control DNA vial. The solution will contain approx. 0.56 ng/µl DNA. After reconstitution, store the solution at 2-8°C.

3. For labeling using $^{32}$P-dATP, prepare 10% (v/v) trichloroacetic acid (TCA) containing 2% (w/v) disodium pyrophosphate and 5% (v/v) trichloroacetic acid (TCA) containing 2% (w/v) disodium pyrophosphate. Chill the solution containing 10% TCA on ice until needed.

**Procedures**

**I. Standard Labeling with $^{32}$P-dATP**

A. Labeling Reaction

1. Prepare 10 ng-1 µg linearized template DNA in a total volume of 45 µl of 1X TE (see Note 1).

2. Denature the template DNA by heating at 95°C for 3 minutes and then immediately placing on ice for 2 minutes.

3. Reconstitute one of the All-In-One random prime reaction tubes by adding the 45 µl denatured template DNA.

4. Add 5 µl $^{32}$P-dATP (10 µCi/µl; 3,000 Ci/mmol) to the tube. Mix briefly.

5. Incubate at 37°C for 15 minutes.

6. Stop the reaction by adding 2 µl of 0.2 M EDTA, pH 8.0. The prepared probe is ready for use at this point.

   Note 1. The DNA may be prepared in a smaller volume. However, note that the final volume in the All-In-One random prime reaction tube should be 50 µl.

B. Determine the Precipitable Radioactivity

1. Dilute a 5 µl aliquot of the labeled DNA mixture with 95 µl of 10 mM EDTA.

2. Place a 5 µl aliquot of the diluted DNA mixture from Step 1 into a tube containing 25 µg of carrier tRNA.

3. Add 1 ml of ice cold 10% (v/v) trichloroacetic acid containing 2% (w/v) disodium pyrophosphate.

4. Invert to mix and centrifuge briefly.

5. Incubate on ice for 15 minutes.

6. Filter reaction onto a glass fiber filter disk using a vacuum manifold.

7. Wash the filter disk five times with 2 ml of 5% (v/v) trichloroacetic acid containing 2% (w/v) disodium pyrophosphate.

8. Remove filter and dry by air or under a heat lamp.

9. Place filter in scintillation cocktail and count in a liquid scintillation counter to determine the precipitable radioactivity (P).

C. Determine the Total Radioactivity

Spot a second 5 µl aliquot of the diluted DNA mixture from Step B1 onto another glass fiber filter disk and dry without the intervening TCA/disodium pyrophosphate washes. Count in a liquid scintillation counter to determine the total radioactivity (T).

D. Determine the Specific Activity

Calculation for Specific Activity (SA) in dpm/μg:

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

μCi = μCi of dATP in reaction

F = Fraction of input label incorporated into DNA: Precipitated Samples (P)/Total Counts(T)

$D_i$ = Mass of input template DNA (ng)

S = Specific activity of dATP (Ci/mmol or μCi/nmol)

$2.2 \times 10^9$ = Factor to convert to dpm from μCi

$1.3 \times 10^3$ = Average molecular weight of the four dNMP's (4x325)

Using this procedure, a specific activity of $1 \times 10^9$ dpm/μg control DNA template is obtained within 15 minutes at 37°C. The procedure labels 25 ng of control DNA with 50 μCi of $^{32}$P-dATP (3,000 Ci/mmol).

**II. Labeling with biotin, digoxin, or fluorescein modified nucleotides**

The following protocol is designed to label 25 ng of denatured DNA using biotin-7-dATP.

1. Prepare 10 ng-1 μg linearized template DNA in a total volume of 45 μl of 1X TE.
2. Denature the template DNA in a microcentrifuge tube by heating for 2-3 minutes to 95°C, then immediately place on ice for 2 minutes.
3. Reconstitute one of the All-In-One random prime reaction tubes by adding the 45 μl denatured template DNA.
4. Add 5 μl of 1 mM biotin-7-dATP for a final concentration of 0.1 mM.
5. Mix gently and incubate at 37°C for 1 hour.
6. Stop the reaction by adding 2 μl of 0.2 M EDTA, pH 8.0. The prepared probe is ready for use at this point.

**III. Labeling DNA in low melting point agarose**

1. The DNA fragment to be labeled is carefully excised from a 1% low-melting agarose gel and transferred to a 1.5 ml tube.
2. Add deionized water in the ratio of 3 ml per g of agarose gel and heat the tube for 7 minutes at 100°C to melt the gel and denature the DNA.
3. Cool the solution to 37°C. The DNA/agarose mixture may now be used for labeling as described in the previously described labeling procedures.

**Results**

Procedural notes

1. If plasmid DNA is to be labeled it must be linearized first.
2. This procedure was optimized for labeling 25-50 ng of DNA. For labeling larger quantities (e.g. 1 μg), lower specific activities should be expected.
3. DNA probes labeled by this procedure can be used in blot hybridizations without removing the unincorporated nucleoside triphosphate. The labeled probe may be removed by ethanol/ammonium acetate precipitation (Add 1/2 volume of 7.5 M ammonium acetate and 2 volumes of 100% ethanol to the mixture. Centrifuge and repeat the precipitation step once).

**References**

1. Feinberg, A.P. and Vogelstein, B., Anal. Biochem., **132**, 6 (1983)
2. Feinberg, A.P. and Vogelstein, B., Anal. Biochem., **132**, 266 (1984)