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SIGMA®

ProductInformation

ALL-IN-ONE™
NICK TRANSLATION DNA LABELING MIX (-dCTP)

Product No. **N9155/N8530**
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TECHNICAL BULLETIN

Product Description

Sigma's All-In-One™ Nick Translation DNA Labeling Mix is based on the combination of DNase I and *E. coli* DNA polymerase I. In the double stranded DNA sample (template), the action of DNase I will introduce randomly distributed nicks, which are then "filled in" by the 5'-3' polymerase activity of the Klenow fragment of DNA polymerase I. As this happens, the 5'-3' exonuclease activity of the Klenow fragment will propagate the "nicks" in the direction of synthesis[1,2]. Deoxycytidine triphosphate (dCTP) 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 Nick Translation reaction tube is ready for use after reconstitution with 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

X **Nick Translation Reaction Tubes**
Product No. N8530 (1.5 ml tubes) or
Product No. N9155 (0.2 ml tubes)
Each reaction tube contains dATP, dGTP, TTP, DNase I, and Klenow fragment of DNA polymerase I, lyophilized with reaction buffer

X **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
- Biotin labeled dCTP, Product No. B3154, or α–^{32}P, digoxin, or FITC labeled dCTP
- Glass fiber filter, >2.3 :m retention, or equivalent, e.g. Product No. F5019
- Molecular biology grade 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, 10 mg/ml, Product No. R8508
- Vacuum manifold, Product No. M2536

Precautions

Sigma's All-In-One Nick Translation DNA Labeling Mix is for laboratory use only. Not for drug, household or other uses. If the sample DNA is to be 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. Prepare 1X TE buffer. If using 100X TE buffer, dilute the concentrate 1:100 with molecular biology grade water to prepare 1X TE buffer.
2. Add 56.25 µl of 1X TE buffer to the control DNA vial. The solution will contain approx. 2.2 ng/µl DNA. After reconstitution, store the solution at 2-8 °C.
3. For labeling using ^{32}P-dCTP, prepare 10% (w/v) TCA containing 2% (w/v) disodium pyrophosphate and 5% (w/v) TCA containing 2% (w/v) disodium pyrophosphate. Chill both solutions on ice until needed.

Procedures

I. Standard Labeling with ^{32}P-dCTP

A. Labeling Reaction

1. Prepare 10 ng-1 µg template DNA in a total volume of 45 µl of 1X TE (see Note).
2. Reconstitute one of the All-In-One Nick Translation reaction tubes by adding the 45 µl template DNA.
3. Add 5 µl ^{32}P-dCTP (10 µCi/µl; 3,000 Ci/mmol) to the tube. Mix briefly.
4. Incubate at 15 °C for 30 minutes.
5. Stop the reaction by adding 2 µl of 0.5 M EDTA. The prepared probe is ready for use at this point.

   Note. The DNA may be prepared in a smaller volume. However, note that the final volume in the All-In-One Nick Translation reaction tube should be 50 :l. Adjust the volume with water if using a small volume of DNA sample (template).

B. Determination of Precipitable Radioactivity

1. Dilute a 5 µl aliquot of the labeled DNA mixture with 95 µl of molecular biology grade water or TE buffer.
2. Place a 5 µl aliquot of the diluted DNA mixture from Step 1 into a tube containing 25 µg (2.5 µl) of carrier tRNA.
3. Add 1 ml of ice cold 10% (w/v) TCA 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 ice cold 5% (w/v) TCA containing 2% (w/v) disodium pyrophosphate. After washing, rinse the filter with 1 ml of absolute ethanol.
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. Determination of 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:

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

µCi = µCi of dCTP 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 dCTP (Ci/mmol or µCi/nmol)

2.2×10^9 = Factor to convert to dpm from µCi

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

Using this procedure, a specific activity of 1×10^8 dpm/:g control DNA template is obtained within 30 minutes at 15°C. The procedure labels 100 ng of control DNA with 50 :Ci of ^{32}P-dCTP (3,000 Ci/mmol).

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

The following protocol is designed to label 1 µg of template DNA using biotin-11-dCTP.

1. Prepare 1 µg template DNA in a total volume of 45 µl of 1X TE.
2. Reconstitute one of the All-In-One Nick Translation reaction tubes by adding the 45 µl template DNA.
3. Add 5 µl of 1 mM biotin-11-dCTP for a final concentration of 0.1 mM.
4. Mix, gently and incubate at 15 °C for 1 hour.
5. Stop the reaction by adding 2 µl of 0.5 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 point agarose gel and transferred to a 1.5 ml tube.
2. Add deionized water in the ratio of 3 ml per gram 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 labeling procedures.

Results

Procedural notes

1. The radioactive procedure was optimized for labeling 25-50 ng of DNA. For labeling larger quantities (e.g. 1 µg), lower specific activities should be expected.
2. DNA probes labeled by this procedure can be used in blot hybridizations without removing the unincorporated nucleotides. If desired, the unincorporated nucleotides may be removed by precipitating the labeled DNA using ethanol/ammonium acetate (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. Klett, R.P. *et al*., Proc. Natl Acad Sci USA, **60,** 946-950 (1968)
2. Rigby, P.W.J., *et al.*, J. Mol. Biol., **113,** 237-251 (1977)