AuPreP Random Primer DNA Labeling Mix System

for 25 labeling assays

Cat No. AUP-RPLM-25

The AuPreP Random Primer DNA Labeling Mix System is a specially designed premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides.

System Details:

The **AuPreP Random Primer Labeling Mix System** is based on the hybridization of oligonucleotides of all possible sequences to the denatured template DNA to be labeled. The complementary DNA strand is synthesized by a "klenow" fragment of DNA Polymerase I, using the random oligonucleotides as primers. By substituting a radiolabeled nucleotide for a non-radioactive equivalent in the reaction mixture, the newly synthesized complementary DNA is made radioactive.

The labeling mix system is a specially developed reaction mixture for enhanced convenience and performance. The reaction mixture contains random oligonucleotides, a Klenow fragment of DNA Polymerase I, dATP, dGTP, dTTP and a reaction buffer concentrate. The DNA labeling mix allows the labeling of the template DNA to a specific activity of $2x10^9$dpm/µg after only 10 minutes of incubation. This rapid labeling is accomplished with the use of the Klenow fragment, which lacks 5′-3′ exonuclease activity, and by the use of nonamer primers giving more efficient priming from the template at 37ºC. The labeling mix method enables the labeling of small amounts of DNA (10-20ng), such as restriction fragments isolated from gels. Fragments can be labeled directly in low melting temperature agarose gel slices. The labeled probes are used in various hybridization techniques, such as Southern and Northern blots, in-situ hybridization and screening of gene libraries.

System Components:

One vial containing 100µl DNA labeling mixture for 25 labeling assays.

Storage and Stability: The premixed Solution should be stored at -20ºC. Avoid repeated changes in the solution temperature.

System Protocol

Add 10-25ng of template DNA to be labeled to a specific activity of $2x10^9$ dpm/µg. The mixture is designed for use with (alpha^{32} P) dCTP with a specific activity of 3000 ci/mmol.

Standard Labeling Procedure

Add 10-25ng of template DNA to be labeled and sterile double distilled water to a final volume of 11µg in a microcentrifuge tube.

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Denature the DNA sample by heating to 95-100°C for 5 minutes, and then chill quickly on ice for 5 minutes.

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Mix on ice:

- 11 µl (10-25ng) of denatured DNA.
- 4 µl of Labeling Mix Solution.
- 5 µl of (alpha^{32} P) dCTP (3000 ci/mmol).

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Incubate at 37°C for 10 minutes.

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Stop the reaction by adding 2µl of 0.2M EDTA (pH =8), or by heating to 65°C for 7 minutes.

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For use in hybridization, denature the labeled DNA by heating to 95°C for 5 minutes and then cool on ice.

Important Notes:

- *A probe with specific activity above 1x10^9 dpm/µg can be obtained after 3 minutes of incubation. The maximum probe specific activity is obtained after 10-20 minutes of incubation.*
- *Less than 10-20ng DNA can also be labeled, but the maximal incorporation may be achieved only after 30-60 minutes.*
- *DNA fragment in low melting temperature agarose can be used directly in the reaction without the removal of the agarose (see Appendix I).*
- *Removal of unincorporated nucleotides is not necessary, but sometimes it is desirable for reducing the background during hybridization. When required, the probes can be purified by gel filtration (Sephadex G-50) or by ethanol precipitation.*
- *Increasing the amount of template DNA above 25ng will reduce the specific activity of the labeled DNA.*

Appendix I: Labeling of DNA fragments in low melting temperature agarose

I After agarose gel electrophoresis, cut out a slice of the gel containing the target DNA fragment.

II Add 3ml distilled sterilized water for each gram of the gel slice.

III Place in boiling water for 10 minutes to melt the gel and to denature the DNA. Keep at 37°C until required.

IV The DNA solution can be used directly in the reaction as template DNA (proceed from Step 1 of the standard labeling procedure).

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References

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