Non-radioactive Random-primed DNA Labeling with Klenow Fragment, exo-

Protocol

This protocol is for Non-radioactive Random-primed DNA Labeling with Klenow Fragment, exo-.

1. Prepare the following reaction mixture:

DNA template	10 µL (100 ng – 1 µg)
10x reaction buffer for Klenow Fragment, exo-	5 µL
6.0 A_{260} units/mL (100 µM) Random Hexamer Primer	12.5 µL
Water, nuclease-free	to 39 µL
Total volume	**39 µL**

2. Incubate the mixture in a boiling water bath for 5-10 minutes and then chill on ice.
3. Add:

3 dNTP Mix, 1 mM each (without the dTTP)	5 µL (0.1 mM final concentration)
dTTP	3.25 µL (0.065 mM final concentration)
Biotin-11-dUTP*, 1 mM	1.75 µL
Thermo Scientific Klenow Fragment, exo- (Cat #EP0421, #EP0422)	1 µL (5 U)
Water, nuclease-free	to 20 µL
Total volume	**50 µL**

* Fluorescein-12-dUTP, DIG-dUTP or Aminoallyl-dUTP can also be used with the same protocol.

4. Incubate the reaction mixture at 37 °C for 1 hour. Add 1 µL 0.5 M EDTA, pH 8.0 to stop the reaction.
5. Remove 1 µL of the reaction mixture and determine the percentage of label incorporated.
6. Optionally, purify by using Sephadex G-50 or Bio-Gel P-60 or size-cutoff appropriate Thermo Scientific GeneJET nucleic acid purification kit.

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