BioArray™
Terminal Labeling Kit with Biotin-ddUTP for DNA Probe Array Assays

25 labeling reactions

Cat. No. 42630

For Research Use Only

INTRODUCTION

The ENZO ***BioArray***™ **Terminal Labeling Kit with Biotin-ddUTP** has been developed for DNA probe array assays. This method uses Bio-ddUTP and terminal deoxynucleotide transferase to catalyze the addition of a single biotin-ddUMP to the 3'-OH terminus of an amplified and fragmented target DNA molecule. A standard reaction will label up to 100 picomoles (equivalent to 1 μg of a 30-nucleotide sequence). When DNA fragments are labeled at the 3'-OH terminus, the sequence bias that occurs with either nick translation or random priming is eliminated.

REAGENTS PROVIDED

The ENZO ***BioArray***™ **Terminal Labeling Kit with Biotin-ddUTP** contains sufficient reagents for 25 labeling reactions of 100 picomoles of fragmented DNA, corresponding to 25 x 1μg of a 30-nucleotide sequence.

Biotin-ddUTP (100X), 25 μl

5X Reaction Buffer, 500 μl
Potassium cacodylate buffer, pH 7, containing β-mercaptoethanol and stabilizer

10X $CoCl_2$ Solution, 250 μl

Terminal Deoxynucleotide Transferase (50X), 50 μl
in storage buffer

WARNING: *The **5X Reaction Buffer** and **Terminal Deoxynucleotide Transferase** contain potassium cacodylate which contains arsenic. It is toxic. Use gloves during the handling of these reagents. Dispose of these reagents and the individual labeling reaction waste materials according to local regulations.*

REAGENTS AND MATERIALS REQUIRED

Preparation and Analysis of Labeled DNA Fragments
- 37°C Water Bath
- Agarose gel (*e.g.,* 4%) - optional
- UV Transilluminator- optional

Termination of Labeling Reaction
- 0.2 M EDTA, pH 8

STORAGE

Upon receipt, store all reagents at -20°C, in a freezer that is not self-defrosting.

PROCEDURE FOR TARGET LABELING

1. To label amplified and fragmented target DNA, add the following reagents (in the indicated order) to a microcentrifuge tube, keeping the tube at **room temperature** while additions are made:

Reagent	Volume
5X Reaction Buffer	20 μl
10X $CoCl_2$	10 μl
Amplified and fragmented target	variable (as required from target preparation protocol)
Biotin-ddUTP	1 μl
Terminal Deoxynucleotide Transferase	2 μl
Distilled or deionized water	variable (to give a final reaction volume of 100 μl)
Total Volume	**100 μl**

2. Carefully mix the reagents in the tube and collect the mixture in the bottom of the microcentrifuge tube by brief (5 seconds) microcentrifugation.
3. Incubate the tube for **15** minutes in a 37°C water bath. The reaction may be allowed to proceed for up to 1 hour.
4. If desired, transfer the tube to ice water (2-4°C), remove 2 μl and analyze on an agarose or acrylamide gel.
5. Stop the reaction by adding 5 μl of 0.2 M EDTA.
6. Store the labeled DNA fragments at -20°C. Do not freeze and thaw repeatedly.

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