INSTRUCTIONS

North2South™ Biotin Random Prime Labeling Kit

17075

0810.4

Number	Description
17075	**North2South Biotin Random Prime Labeling Kit,** sufficient reagents for 10 biotin-labeling reactions, each with 100ng DNA.
	Kit Contents:
	Heptanucleotide mix (5X), 100µL
	dNTP mix (5X), 1mM dATP, 1mM dTTP, 1mM dGTP, 1mM dCTP, 80µL
	Reaction Buffer (10X), 50µL
	DNA Polymerase, Klenow fragment (3´-5´ exo-), 5 units/µL, 10µL
	Control DNA, 250ng/µL, 5µL
	EDTA, 500mM, pH 8.0, 1mL
	Nuclease-free water, 1mL
	Ammonium acetate (NH_4OAc), 5M, 1mL
	Biotin-11-dUTP, 20µL
	Storage: Upon receipt store all components at -20°C in a non-defrosting freezer. Product is shipped with dry ice.

Introduction

The Thermo Scientific™ North2South™ Biotin Random Prime Labeling Kit provides the components and procedure to successfully prepare biotin-labeled DNA probes for use in Southern blotting and other DNA hybridization methods involving streptavidin detection systems. The random prime labeling method is based on the procedure of Feinberg and Vogelstein,[1,2] wherein random heptanucleotides containing all possible sequences anneal to a denatured DNA template and act as primers for complementary strand synthesis by DNA Polymerase (Klenow fragment, 3´-5´ exo$^-$). Inclusion of biotinylated nucleotides in the reaction mixture ensures that the newly synthesized DNA strands are labeled with biotin. This protocol yields biotin-labeled DNA of high activity for use as probes in experiments using the North2South Chemiluminescent Hybridization and Detection Kit (Product No. 17097).

Additional Materials Required

- Heating block
- 1X TE Buffer (10mM Tris•HCl, 1mM EDTA, pH 8.0)
- Ethanol
- Linearized template DNA, i.e., the DNA to be labeled

Procedure for Biotin Random Prime Labeling

A. Prepare Complete 5X dNTP mix

Before first use of the kit, prepare a complete 5X dNTP mix by adding entire contents of the Biotin-11-dUTP (20µL) to the tube of 5X dNTP mix (80µL). This 100µL complete stock solution will henceforth be referred to simply as '5X dNTP mix'. After first use, store the prepared 5X dNTP mix at -20°C with the other kit components. In order to estimate your experimental probe labeling efficiency the control DNA will need to be labeled. Once labeled, this probe can be stored at -20°C for use in future probe labeling assessments.

B. Perform Labeling Reaction

1. In a microcentrifuge tube, dilute ~100ng of linear DNA for labeling (template DNA) to a final volume of 24µL in nuclease-free water. For the positive control, dilute 0.5µL of control DNA (125ng) to 24µL.
2. Add 10µL of heptanucleotide mix and denature the DNA template by boiling the tube for 5 minutes.
3. Quickly freeze the denatured DNA solution by placing the tube in a dry ice/ethanol bath for 5 minutes.
4. Thaw DNA solution at 4°C and briefly centrifuge to collect liquid in the bottom of the tube. Place the tube on ice.
5. In the order stated, add the following components to the prepared sample solution on ice:
   - 10µL of 5X dNTP mix
   - 5µL of 10X Reaction Buffer
   - 1µL Klenow fragment
   (Final volume = 50µL)
6. Mix contents by flicking or briefly vortexing the tube. Centrifuge briefly to collect liquid in the bottom of the tube.
7. Incubate labeling reaction for 60 minutes at 37°C.
8. Inactivate the enzyme by adding 2µL of 500mM EDTA, pH 8.0.

C. Ethanol Precipitation to Remove Unincorporated Nucleotides

Note: As an alternative to the ethanol precipitation method described here, size exclusion chromatography with an appropriate gel filtration column may be used to remove unincorporated nucleotides.

1. Adjust the reaction to contain 0.5M ammonium acetate. For example, add 5µL of 5M NH_4OAc to a 50µL labeling reaction and mix well.
2. Add 2 volumes of 100% ethanol and mix well. For example, add 110µL to the 55µL volume from Step 1.
3. Chill tube at -20°C for 15 minutes.
4. Centrifuge tube at 4°C for 30-60 minutes at maximum speed (> 10,000 × *g*).
5. Carefully aspirate to remove (and discard) the supernatant.
6. Wash the DNA pellet once by adding ice-cold 70% ethanol and centrifuging for 30-60 minutes at maximum speed. Carefully remove and discard the supernatant.
7. Dissolve the DNA pellet in 100µL of 1X TE or nuclease-free water and store at -20°C. For long-term storage, prepare single-use aliquots and store at -70°C.

Procedures for Estimating Labeling Efficiency

Labeling efficiency can be determined by dot blotting using a hand spotting method. The following dot blot protocol was developed for use with the detection reagents in our **Chemiluminescent Nucleic Acid Detection Module (Product No. 89880).**

Additional Materials Required

- Positively-charged nylon membrane (e.g., Biodyne™ B Membrane, Product No. 77016)
- TE Buffer: 10mM Tris•HCl, 1mM EDTA, pH 8.0
- Microcentrifuge tubes and microcentrifuge

- UV lamp or crosslinking device equipped with 254nm bulbs or 312nm transilluminator
- Chemiluminescent Nucleic Acid Detection Module (Product No. 89880)
- X-ray film or CCD camera

D. Dot Blot by Hand Spotting

1. Hydrate/equilibrate a positively-charged nylon membrane in TE Buffer for at least 10 minutes.
2. Pipette 20µL TE Buffer into a micro centrifuge tube labeled #1 and 10µL into micro centrifuge tubes labeled #2-7 for the control reaction and each experimental sample.
3. Prepare dilutions of the labeled DNA from both the control DNA and your experimental probe with TE. Dilute the labeled samples 10-fold in TE Buffer by adding 2µL of the stated samples into the #1 tubes and serially diluting the sample serially into the other tubes using 10µL per dilution.
4. Place the equilibrated membrane onto a clean, dry paper towel. Allow excess buffer to absorb into the membrane, but do not allow the membrane dry out.
5. Spot 2µL of samples and standards onto the hydrated membrane. Allow the samples to absorb into the membrane.

E. UV Crosslink the Dot Blot

1. Immediately UV crosslink the membrane by one of the following three methods:

- **Option 1**: Crosslink at 120mJ/cm^2 using a commercial UV-light crosslinking instrument equipped with 254nm bulbs (45-60 second exposure using the auto crosslink function).
- **Option 2**: Crosslink at a distance of approximately 0.5cm from the membrane for 5-10 minutes with a hand-held UV lamp equipped with 254nm bulbs.
- **Option 3**: Crosslink for 10-15 minutes with the membrane face down on a transilluminator equipped with 312nm bulbs.

2. Continue with the detection and analysis immediately or store the membrane dry at room temperature until the detection protocol can be performed.

F. Detection and Analysis

Detect the spotted standards and samples using the procedure and reagents for the North2South Chemiluminescent Hybridization and Detection Kit (Product No. 17097) or the Chemiluminescent Nucleic Acid Detection Module (Product No. 89880). To determine the labeling efficiency, compare spot intensities of the sample lanes to those of the labeled control DNA.

- If using X-ray film to document results, compare the row of sample spots to the row of labeled control DNA spots to estimate relative efficiency. Densitometry of the film can also be performed.
- If using a cooled CCD camera to document results, spot densitometry can be performed (choose a dilution that is within the linear exposure range) assess the labeling efficiency of the unknown samples.

Compare the spot intensities of experimental labeled probes to the labeled Control DNA. The intensity of your experimental probe should be approximately 50% of the labeled control DNA to yield satisfactory hybridization results. If the probe signal is weaker than this it will be difficult to detect signals from low abundance species. The amount of probe required for downstream applications must be empirically determined.

Troubleshooting

A poor quality starting DNA template is the most likely reason for poor probe synthesis labeling when compared to that of the purified control DNA supplied in the kit. In some cases, using less than the recommended starting DNA template is better because it reduces the concentration of inhibitors in the reaction. Alternatively, the DNA template may require further purification before use in the labeling reaction.

Related Thermo Scientific Products

17097	**North2South Chemiluminescent Hybridization and Detection Kit**
89880	**Chemiluminescent Nucleic Acid Detection Module** (this module includes the same components as Product No. 17097, except the hybridization and stringency wash buffers.)
20148	**LightShift™ Chemiluminescent EMSA Kit**
17295	**North2South Chemiluminescent Substrate for HRP,** 100mL
77016	**Biodyne B Nylon Membranes, 8 × 12cm,** 25 sheets, positively charged membrane with 0.45µm pore size

Cited References

1. Feinberg, A.P. and Vogelstein, B. (1983). *Anal Biochem* **132:**6.
2. Feinberg, A.P. and Vogelstein, B. (1983). *Anal Biochem* **137:**266.

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