Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye *Optimized for Labeling 100-300 ug DNA/RNA*

Catalog number: 17960
Unit size: 1 mg

Component	Storage	Amount (Cat No. 17960)
Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye *Optimized for Labeling 100-300 ug DNA/RNA*	Freeze (< -15 °C), Minimize light exposure	1 vial (1mg)

OVERVIEW

Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye is a key member of our enabling Helixyte™ nucleic acid labeling and conjugation technology. The labeling/conjugation of a tag/hapten to nucleic acids has been very challenging due to the lack of reactive moieties in nucleic acid molecules. Thymine and guanosine have been often explored for nucleic acid conjugations, e.g., photo-crosslink (to thymine by psoralens) or bromination/Ulysis labeling of guanosine. However, these existing conjugation techniques are either tedious, ineffective or require stringent conditions with low yields and are thus not suitable for routine lab use. Under the similar conditions, our Helixyte™ nucleic acid labeling and conjugation technology is much easier to use with significantly higher yield. Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye provides a unique method to attach the iFluor® 594 fluorophore to nucleic acids via a simple mixing step. The labeling reagent readily reacts with the N7 of guanine to form a stable covalent bond. The labeling procedure is simple and fast with a high production yield. The separation of the labeled nucleic acids from the unreacted dye can be accomplished with a simple ethanol precipitation, a spin-column or dialysis. The resulting labeled DNA/RNA probes have bright red and stable fluorescence that can be easily detected with Texas Red filter set. They can be used for dot, Northern and Southern blots, RNA and DNA in situ hybridization, multicolor fluorescence in situ hybridization (mFISH), comparative genome hybridization (CGH) or microarray analysis etc.

AT A GLANCE

Protocol Summary

1. Combine DNA with the Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye stock solution.
2. Incubate for 1 hour at 37°C.
3. Purify the conjugate as required for downstream applications.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Important

Before opening the vial, thaw Helixyte™ iFluor® nucleic acid labeling dye at room temperature. Briefly centrifuge to collect the dried pellet.

Prepare a Helixyte™ iFluor® Nucleic Acid Dye Stock Solution

1. Add 70 µL of DMSO to the Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye vial to prepare a 10 mM stock solution.

   Note: It is recommended to divide any unused stock solution into single-use aliquots. Store the aliquots at ≤-20 °C and protect them from light. Avoid repeated freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

Protocol

1. Prepare the labeling reaction according to the specifications in table 1 below.

   Table 1. Standard Nucleic Acid Labeling Reaction.

Components	Volume added to reaction	Final Concentration
DNA (1 mg/mL)	2 to 5 µL	2 to 5 µg
Helixyte™ iFluor® 594 Nucleic acid Labeling Dye stock solution	0.5 µL	50 µM
TE Buffer (pH 8 to 8.5)	Add sufficient buffer to adjust the volume to 100 µL	

   Note: This DNA:Dye ratio results in labeling efficiencies that are appropriate for most applications. The amount of Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye or the reaction incubation time can be adjusted to modify the labeling density as per the application requirements. The DNA-to-dye ratio must be optimized to achieve a higher labeling ratio.

2. Incubate the reaction at 37°C for 1 hour, protected from light.

   Note: After 30 minutes of incubation, briefly centrifuge the reaction to minimize the effects of evaporation and maintain the appropriate concentration of the reaction components.

   Note: Alternatively, the reaction can be incubated at room temperature for 2 hours. For the best labeling condition, we recommend incubating at 37°C.

3. After incubation, the labeling mix can be purified to remove any free labeling dye. Refer to the "Purification of labeling mix with alcohol precipitation" section below for instructions.

Purification of Labeling Mix with Alcohol Precipitation

1. Add 0.1 volume (10 uL) of 5M sodium chloride and 2 - 2.5 volumes of ice-cold 100% ethanol (250 uL) to the reaction. Mix well and place at ≤ -20°C for at least 30 minutes.
2. Centrifuge at full speed (>14,000 x g) in a refrigerated micro centrifuge for 15-30 minutes to pellet the labeled nucleic acid. Once pelleted, carefully remove the ethanol with a micropipette.

Do not disturb the pellet.

Note: Small nucleic acid quantities can be difficult to visualize. Mark and orient the precipitate-containing tubes in the microfuge such that the pellet will form in a predetermined place.

3. Wash the pellet once with 500 µL of room temperature 70% ethanol. Centrifuge at full speed for an additional 15-30 minutes.

4. Remove all traces of ethanol with a micropipette. **DO NOT** allow the sample to dry longer than 5 minutes as the pellet may become difficult to resuspend.

5. Resuspend the labeled DNA with ~ 30 µL sterile water.

6. Store the purified, labeled nucleic acid for long-term storage or put on ice for immediate use.

EXAMPLE DATA ANALYSIS AND FIGURES

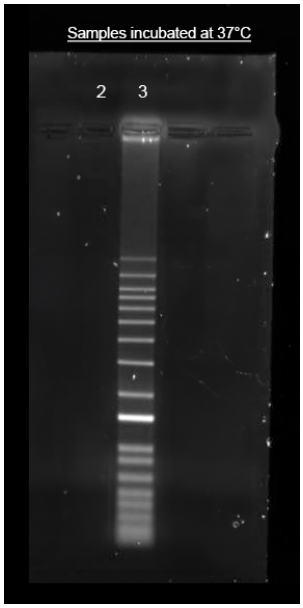

Figure 1. Direct labeling of nucleic acid using Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye. DNA ladder was labeled with 50 µM of Helixyte™ iFluor® 594 Nucleic Acid Labeling Dye (Lane 3) and analyzed alongside unlabeled DNA (Lane 2) on 1% agarose DNA gel using gel electrophoresis.

DISCLAIMER

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