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829-05565

Storage: -20°C

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M13 Forward (-29) IRDye® 800 Primer

For LI-COR DNA Analysis Systems

Dilution Procedure

Add 1000 µl sterile ice-cold 1XTE Buffer to the 1.0 nmol of lyophilized primer. A tube of 1XTE Buffer is included with your primer order. Mix well until all of the primer is dissolved (undissolved primer should be easily visible as a dark green pellet). Aliquot into 20 sterile microcentrifuge tubes at 50 µl/tube.

This dilution will produce a primer solution with a 1.0 µM (1.0 pmol/µl) concentration. This primer solution may be used directly in LI-COR sequencing protocols.

1XTE Buffer Solution

The 1XTE included with the primer is 10 mM Tris, 1 mM EDTA, pH = 7.4 (± .2). Additional 1XTE can be made by diluting 10 µl of 100XTE Buffer (Sigma Chemical Company, Catalog #T9285) to a 1 mL total volume with sterile distilled water. Store at 4°C.

Measuring Concentration

The concentration of the primer solution may be verified by measurement of a dilution of the final solution on a UV/Vis absorption spectrophotometer equipped with a microcell. Since EDTA interferes with measurement of absorbance at 260 nm, the concentration should be determined based on the IRDye® 800 peak at 795 nm. The molar absorptivity of IRDye 800 at 795 nm in aqueous solution is 270,000. The concentration may be calculated using the following formula:

$$\text{(Absorbance at 795 nm)} \times \text{Dilution Factor} / (270,000 \times$$

$$\text{Cell path length (cm)}) = \text{Molar concentration of the original solution}$$

Example: If, using a 1 cm path length cuvette, the absorbance at 795 nm equals 0.15 for a 1:2 dilution of the final primer solution, the concentration of the original solution can be determined as follows.

$$(0.15 \times 2) / (270,000 \times 1) = 1.1 \times 10^{-6} \text{ M} = 1.1 \mu\text{M}$$

Storage Conditions

Minimize exposure to light. Store tubes at -20°C in a light-tight container or foil shipping bag. Minimizing freeze/thaw cycles may help preserve the integrity of the fluorescent primer.

Sequence: 5'- CAC GAC GTT GTA AAA CGA C- 3'

Tm: 58.1°C

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