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829-05571

Storage: -20°C

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M13 Reverse IRDye® 700 Budget 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 blue 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 700 peak at 685 nm. The molar absorptivity of IRDye 700 at 685 nm in aqueous solution is 170,000. The concentration may be calculated using the following formula:

$$\frac{(\text{Absorbance at 685 nm}) \times (\text{Dilution Factor})}{(170,000 \times \text{Cell path length (cm)})} = \text{Molar concentration of the original solution}$$

Example: If, using a 1 cm path length cuvette, the absorbance at 685 nm equals 0.10 for a 1:2 dilution of the final primer solution, the concentration of the original solution can be determined as follows:

$$(0.10 \times 2) / (170,000 \times 1) = 1.2 \times 10^{-6} \text{ M} = 1.2 \mu\text{M}$$

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'- GGA TAA CAA TTT CAC ACA GG -3'

Tm: 54.1°C

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