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**829-05566**

Storage: -20°C

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4647 Superior Street • P.O. Box 4000  
Lincoln, Nebraska 68504 USA  
North America: 800-645-4267  
International: 402-467-0700  
FAX: 402-467-0819

LI-COR GmbH Germany, Serving Europe and  
Africa: +49 (0) 6172 17 17 771  
LI-COR UK Ltd. UK, Serving UK, Ireland,  
Scandinavia: +44 (0) 1223 422104  
[www.licor.com](http://www.licor.com)

# M13 Reverse IRDye® 800 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 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:

$$(270,000 \times \text{Cell path length (cm)}) = \frac{(\text{Absorbance (at 795 nm)} \times \text{Dilution Factor})}{\text{Molar concentration of the original solution}}$$

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

**Tm:** 54.1°C

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