



## Hi Fidelity polymerase

| Cat No | Pack size | conc  |
|--------|-----------|-------|
| LT0500 | 500 U     | 5U/ul |
| LT2500 | 2500 U    | 5U/ul |

### Description:

Hi Fi polymerase are thermostable enzymes formulation specifically developed for synthesizes length up to 30 kb and low error rate PCR product.

Hi Fi polymerase synthesizes higher yields of product from genomic DNA, cDNA, bacterial cultures. It is contain a 2.5 hours half life at 96°C and easy to amplify PCR product at G-C rich and secondary structure.

### Reaction Buffer (10x) with MgCl<sub>2</sub>: 25 mM

**storage conditions:** -20°C

### Unit Definition

One unit of Hi Fi DNA Polymerase incorporates 10 nmol of dNTP into acid-insoluble material in 30 min at 74°C.

### Template

Hi Fi Polymerase is suitable for amplifying targets up to 15 kb from the following templates:

Genomic DNA: 10–200 ng

Plasmid DNA : 1–5 ng

cDNA : ~100 ng starting total RNA

Amplification of longer targets (up to 15 kb) may be possible, but may require more template and longer elongation times.

### Primers

Use 0.3  $\mu$ M per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5  $\mu$ M per primer may improve yield.

### Annealing Temperature

The annealing temperature is slightly higher than with typical PCR. The optimal annealing temperature should be ~2°C lower than the T<sub>m</sub> of the primers used. A range of 58–68°C is recommended.

**Extension Time:** As little as 30 seconds per kb is suitable for most targets. Use up to 60 seconds per kb for maximum yield.

### PCR Protocol

The following procedure is suggested as a starting point when using Hi Fi Polymerase in any PCR amplification.

1. Add the following components to an autoclaved micro centrifuge tube at room temperature. Mix of common components to enable accurate pipetting):

| Component          | Volume       |
|--------------------|--------------|
| Hi Fi polymerase   | 0.5-1ul      |
| 10X buffer         | 10 ul        |
| 10mM dNTP          | 2 ul         |
| Primer1 (20 pmol)  | 2-4 ul       |
| Primer2 (20 pmol)  | 2-4 ul       |
| template           | 1-10 ul      |
| ddH <sub>2</sub> O | Up to 100 ul |
| Total              | 100 ul       |

### 1. Program the thermal cycler as follows:

| Step                 | Temperature | Time      | Cycle |
|----------------------|-------------|-----------|-------|
| Initial denaturation | 94-96°C     | 0.5-2mins | 1     |
| Denaturation         | 94-96°C     | 0.2-2mins | 15-30 |
| Annealing            | 50-68       | 0.2-2mins |       |
| Extension            | 68-75       | 1min/1kb  |       |
| Final extension      | 68-75       | 1-10mins  | 1     |

### Step

After cycling, maintain the reaction at 4°C. Samples can be stored at -20°C until use.

Analyze products using standard agarose gel electrophoresis.

ZymesetBiotek

Address: 4F,No.72-3 Lane 296 Sec 6 Min-Chung East Rd. 114 Taipei (Taiwan)

phone(886)2 26347345 Fax: (886)2 26347346 email: max@zymeset.com