

Description

Hi Fidelity polymerase is a mixture of thermostable enzymes. It is specifically developed to synthesize length of PCR product up to 25 kb and with low error rate. Hi Fi polymerase synthesizes higher yields of product from genomic DNA, cDNA, and bacterial cultures. It has 2.5 hours half life at 96oC and easily amplify PCR product of G-C rich or secondary s t r u c t u r e DNA by adding G-C rich bu f f e r.

storage conditions

-20°C

10X reaction buffer

Buffer containing 25mM MgCl2

Unit description

One unit is defined as the amount of enzyme that will incorporate 10nmole of dNTP into acid-insoluble material in 30 minutes at 74°C. The reaction conditions are: 50mM Tris-HCl pH8.8, 50mM NaCl, 5mM MgCl2, 200uM each of dATP, dCTP, dGTP, dTTP,10ug

activated calf thymus DNA and 0.1mg/ml BSA in a final volume of 50ul.

Dr. C. 分子生物試劑

Template

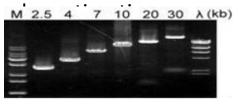
Hi Fidelity Polymerase is suitable for amplifying targets up to 25 kb from the following templates:

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



Hi Fidelity polymerase amplified λ DNA from 2.5k to 30k DNA fragement

Primers

Use 0.3uM per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5uM 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 Tm 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



Program the thermal cycler

| Step | Temperature | Time | Cycle |
|----------------------|-------------|-----------|-------|
| Initial denaturation | 94-95 ºC | 1-3 mins | 1 |
| Denaturation | 94-95 ≌C | 10-60sec | |
| Annealing | 50-68 ºC | 10-30sec | 25-35 |
| Extension | 72 ºC | 1min/1kb | |
| Final extension | 72 ºC | 1-10 mins | 1 |

IMPORTANT: Annealing temperature should be 2-6°C lower than the primer melting temperature.

Shipping and Storage conditions

Shipping and temporary storage at -20°C and for up to 1 month at room temperature has no detrimental effects on the quality of Hi Fidelity DNA polymerase.