

## ThermoHybrid™ High-Fidelity DNA Polymerase (Fusion)

Products	Cat #	Pack Size
ThermoHybrid™ DNA Polymerase (Fusion)	GC-014-0250	250 u
ThermoHybrid™ DNA Polymerase (Fusion)	GC-014-0500	500 u

### DESCRIPTION

The ThermoHybrid™ unique DNA Polymerase offers robust performance and can be used for all PCR applications. Its unique structure, a novel **Pyrococcus-like** enzyme fused with a processivity-enhancing domain, **increases fidelity and speed**. ThermoHybrid™ DNA Polymerase is an ideal choice for cloning and can be used for long or difficult amplicons. With an **error rate 50-fold lower** than that of Taq DNA Polymerase and **6-fold lower** than that of *Pyrococcus furiosus* DNA Polymerase, ThermoHybrid™ is the **most accurate thermostable polymerase** available. ThermoHybrid™ DNA Polymerase possesses 5'→3' polymerase activity, 3'→5' exonuclease activity and will generate blunt-ended products.

### APPLICATION

- ✓ High-fidelity PCR
- ✓ Cloning
- ✓ Long or Difficult Amplification
- ✓ DHPLC
- ✓ Microarray

### CONCENTRATION

2 units/μl

### UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 75°C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25°C), 100 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mM β-mercaptoethanol, 200 μM each dNTP and 10 μg activated calf thymus DNA in 50 μl.

### 5 x REACTION BUFER

ThermoHybrid™ DNA Polymerase is supplied with 5x ThermoHybrid™ buffer containing 7.5 mM MgCl<sub>2</sub> in the provided 5x concentration.

### STORAGE BUFFER

20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA; 1 mM DTT, 50% glycerol and stabilizers

Store ThermoHybrid™ DNA Polymerase below 0°C, preferably at -20°C, in a constant temperature freezer

SHELF LIFE **18 months from date of receipt if stored at -20°C.**

# General Protocol for amplification

## with ThermoHybrid™ High-Fidelity DNA Polymerase (Fusion)

The optimal reaction conditions for ThermoHybrid™ DNA Polymerase may differ from PCR protocols for standard (Taq-like) DNA polymerases. PCR conditions for ThermoHybrid™ DNA Polymerase is similar in PCR conditions to “Fusion-like” DNA polymerases, e.g. ThermoHybrid™ DNA Polymerase works better at elevated denaturation and annealing temperatures.

PCR reactions should be set up on ice. Prepare a master mix for the appropriate number of samples to be amplified.

**NOTE: It is critical that the ThermoHybrid™ DNA Polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3'→5' exonuclease activity, that can degrade primers in the absence of dNTPs.**

Add and mix the following component on ice:

Component	50 µl reactions	25 µl reactions	Final concentration
PCR grade Water	up to 50 µl	up to 25 µl	
5x ThermoHybrid™ Buffer	10 µl	5 µl	1x
10 mM MIX dNTPs	1 µl	0.5 µl	0.2 mM each
Primers			0.3-0.5 µM each
Template DNA	optionally	optionally	
ThermoHybrid™ polymerase 2 units/µl	0.5-1 µl	0.25-0.5 µl	0.02-0.04 U/µl

\* We recommend the following concentrations of ThermoHybrid™ DNA Polymerase for different length amplicons: up to 4 kb – 1-2U/50 µl ; 4-8 kb – 0.6-1U/50 µl ; 8-12 kb – 0.4-0.6U/50 µl.

Cycling protocol:

Cycle step	2-step amplification		3-step amplification		Cycles
	T°C	Time	T°C	Time	
Initial Denaturation	98°C	30 sec	98°C	30 sec	1
Denaturation	98°C	5-10 sec	98°C	5-10 sec	25-35
Annealing	-	-	Annl. Temp.	10-30 sec	
Extension	72°C	15-30 sec/Kb	72°	15-30 sec/Kb	
Final extension	72°C	5-10 min	72°C	5-10 min	1
	4°C	hold	4°C	hold	