Data sheet

Hot Start Pol

Heat-activatable DNA polymerase for high specificity

Thermus aquaticus, recombinant, *E. coli*

	Cat. No.	Size	Conc.
	HS_100KU	100 kU	5 units/µl
	HS_1000KU	1000 kU	5 units/µl

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20°C, avoid frequent thawing and freezing

Unit definition
One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmoles of dNTP's into an acid-insoluble form in 30 minutes at 72°C using hering sperm DNA as substrate.

Hot Start Pol (red cap)
5 units/µl heat-activatable DNA Polymerase in 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween-20, 0.5% Nonidet P-40, 50% (v/v) Glycerol, pH 8.0 (25°C)

10x Hot start buffer complete (green cap)
200 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, pH 8.5 (25°C)

10x Hot start buffer without $MgCl_2$ (blue cap)
200 mM Tris-HCl, 500 mM KCl, pH 8.5 (25°C)

$MgCl_2$ stock solution (yellow cap)
25 mM $MgCl_2$

Description
Hot Start Pol provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds or when prolonged room-temperature set up is required. The polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup.
The enzyme catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium. It also possesses a 5'→3' polymerization-dependent exonuclease replacement activity but lacks a 3'→5' exonuclease activity.

Activation step
Hot Start Pol requires no prolonged heating or denaturing step. The polymerase inhibiting ligand is quickly released at the increased temperature of thermal cycling.

Recommended PCR assay

50 µl PCR assay		
5 µl	10x Hot start buffer complete	green cap
200 µM	each dNTP	
0.2-1 µM	each Primer	
2-50 ng	Template DNA	
0.2-0.5 µl (1-2.5 u)	Hot Start Pol	red cap
Fill up to 50 µl	PCR grade H_2O	

Optimization of $MgCl_2$ concentration
A concentration of 1.5 mM Mg^{2+} is recommended for most applications. For an individual optimization use the reaction buffer without $MgCl_2$ and add $MgCl_2$ stock solution as shown in the table below.

50 µl PCR assay				
$MgCl_2$ stock.	2 µl	3 µl	4 µl	6 µl
Final $MgCl_2$ conc.	1 mM	1.5 mM	2 mM	3 mM