

## Hot Start Master

### Master Mix of heat-activatable DNA polymerase for high specificity

Ready-to-use mixes for PCR

	Cat. No.	Amount	Size
	PCR-103S	100 reactions	1 ml
	PCR-103L	500 reactions	5 ml

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C, avoid frequent thawing and freezing

Storage at 4°C for up to 3 months possible

#### Description

Hot Start Master contains all reagents required for PCR (except template and primer) in a premixed 5x concentrated ready-to-use solution.

The master mix 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-dimers formed at low temperatures during PCR setup.

#### Activation step

Hot Start Master 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		
10 µl	5x Hot Start Master Mix	red cap
0.2-1 µM	each Primer	
2-50 ng	Template DNA	
Fill up to 50 µl	PCR grade H <sub>2</sub> O	white cap

#### Recommended cycling conditions

Activation step and initial denaturation	94°C	2 min	1x
Denaturation	94°C	30 sec	30x
Annealing <sup>1)</sup>	45-68°C	30 sec	
Elongation <sup>2)</sup>	72°C	30 sec - 4 min	
Final elongation	72°C	2 min	1x

1) The annealing temperature depends on the melting temperature of the primers used.

2) The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kbp is recommended.

#### 5x Hot Start Master (red cap)

Thermally activated DNA polymerase, dATP, dCTP, dGTP, dTTP, reaction buffer with KCl and MgCl<sub>2</sub>, stabilizers

#### PCR grade water (white cap)

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new template DNA and/or primer pair.