HotStart PCR Master Mix

CAT. NO. DP-008-0250, 250 rxns
DP-008-2500, 2500 rxns

APPLICATION

1. Hot start PCR amplification of all various templates or tasks ;
2. designed for amplification of difficult templates, such as GC-rich fragments and microsatellites;
3. primer extension of SNP markers;
4. amplification of genomic DNA targets up to 10 kb with high fidelity, specificity, and sensitivity;
5. high through-put Hot Start PCR with high specificity, sensitivity, and yield;
6. routine diagnostic Hot Start PCR requiring high reproducibility.

DESCRIPTION

HotStart PCR Ready Mix is a 2 x concentrated reagent mix for Hot Start PCR comprising two cold sensitive mutant Taq polymerases complexed with a proprietary Taq monoclonal antibody, which blocks polymerase activity prior to the onset of thermal cycling. This prevents primer-dimers and other artifacts resulting from low-level synthesis from nonspecifically primed sites. The antibodies are quickly inactivated by the increased

temperature of thermal cycling. The monoclonal antibody based Hot Start PCR Ready Mix requires no prolonged heating or denaturing step as do other hot-start methods.

The Hot Start PCR Ready Mix has been well designed for amplification of genomic DNA sequences, particularly for genotyping with multiple primer sets.

COMPOSITION

Taq polymerases, 100 mM Tris-HCl pH 8.75 (at 25°C), 50 mM $(NH4)_2SO4$, 5 mM MgCl2, 0.5% Trinton X-100, 40 µM of each dNTP, Taq monoclonal antibody, and stabilizers.

COMPONENT

Cat# DP-008-0250:

2 x HotStar PCR ready mix	2 x 1.25 ml
ddH_2O	2 x 1.25 ml

Cat# DP-008-2500:

2 x HotStar PCR ready mix	20 x 1.25 ml
ddH_2O	20 x 1.25 ml

STORAGE TEMPERATURE

Recommended to keep at 4°C for immediate use (6 month) and -20°C for long term storage.

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Protocol

1. Place the PCR reaction tubes on ice.
2. Prepare first a template/primer mix according to the volumes given in the table below for different reaction volumes. Mix the template/primer mix and chill on ice.
3. Dispense the corresponding volume of 2 x HotStart PCR Ready Mix (25 µl for a 50 µl reaction) followed by the template/primer mix into each reaction tube. Mix well and chill the tubes on ice until all samples have been prepared.

PCR Reaction Setup for a 50 µl Assay

PCR Volume	ddH_2O	Sense Primer	Antisense Primer	Template DNA	2 x PCR Ready Mix
50 µl	Up to 25 µl	x µl	y µl	z µl	25 µl
Final Concentrations:		200 nM	200 nM	0.2-20 ng	1x

Other variable reaction conditions (temperatures, cycling times, concentrations of template, primers, magnesium and polymerase) have to be optimized empirically for each template/primers combination. Most PCR applications work at the concentration of 2.5 mM Mg^{2+} provided with 1x diluted PCR Ready Mix. Optimal Mg^{2+} concentration higher than 2.5 mM can be adjusted using a separate magnesium solution or by increasing stepwise (10 µl increments for 100 µl reaction) the amount of 2 x HotStart PCR Ready Mix added to the reaction assay. This approach can also be used to improve the product yield in amplification of difficult targets on complex template DNA (see table below).

Standard Temperature cycling Program		
1. 94°C	**4 min**	
2. 94°C **55-65°C** **68°C**	**30-60 sec** **30-60 sec** **1 min (add 1 min per kb target sequence length)**	**25-35 Cycles**
3. 72 °C	**5 min**	
4. 4 °C		

Note:

1. High quality thin-wall PCR tubes, strips, and plates are highly recommended for getting a consistent and duplicable PCR results. This is particularly important when amplification volume is less than 20 µl. Our EU thin-wall tubes can produce the most reliable results and the amplification volume can be as low as 5 µl.
2. The typical annealing temperature is between 55 °C and 72 °C. The optimal annealing temperature could be calculated by "Tm - 5 °C".