CHAI

990 Richard Ave, Suite 110
Santa Clara, CA 95050
www.chaibio.com

support@chaibio.com
+1 (800) 642-4002 Toll-free
+1 (650) 779-5577 International

www.chaibio.com

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GENERAL GUIDELINES FOR PCR

Primers & Probes: Various free software can be found online to design or analyze primers for PCR and qPCR. Primers/probes are generally 18 - 30 nucleotides long with a GC content of 40 to 60%. Optimize primer concentrations using a test range at final concentrations of 0.1 µM to 0.5 µM for both primers. The optimal concentration is one that gives the lowest Cq value. Next, optimize probe concentration using a test range of 0.1 µM to 0.5 µM. The optimal probe concentration is the one that gives the lowest Cq value and the highest fluorescence signal.

Additives: Magnesium is a cofactor necessary for Taq polymerase activity. The magnesium ion concentration in the 1X buffer is 3 mM. More magnesium can be added to further optimize the reaction if needed. Amplification of GC rich templates may be improved by using enhancers like DMSO or formamide to stabilize the template for amplification.

PCR consists of three steps: Denaturation, Annealing and Extension.

Denaturation: Heating the DNA to 95 ºC separates the strand and, in hot start mixes, activates the Taq which is inhibited by an aptamer at low temperatures. Initial denaturing of 2 minutes at 95 ºC is sufficient for most templates. For difficult templates, a longer denaturing such as 2 - 4 minutes at 95 ºC is recommended.

Annealing: The annealing stage allows primers to bind to the DNA. PCR primers copy only a specific sequence of template DNA within a sample. The temperature for annealing depends on the melting temperature (Tm) of the primers. Annealing temperatures can be optimized by performing a gradient PCR starting at 5 ºC below the calculated Tm of the primers. When primers with annealing temperatures above 60 ºC are used, the annealing and extension steps can be combined, creating a two-step PCR protocol.

Extension: The optimal extension temperature is 68 ºC. At this temperature, the Taq polymerase uses the dNTPs in the Master Mix to make new copies of the target DNA. Extension times are generally 1 minute/kb. Each cycle copies the target DNA available in the sample and effectively doubles it. A final extension time of 5 minutes at 68 ºC is recommended.

Number of Cycles: 30 to 40 cycles generally give sufficient yield for amplification products. 40 cycles may be used for detection of low copy targets.

Refrigeration: Storing the reaction products at 4 ºC after the final extension step can minimize polymerase activity that may happen at higher temperatures. Store at -20 ºC for long-term storage.

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PCR Master Mix with **Hot Start** DRY

Catalog #: R02160

For research use only

Store at -20 to 4 ºC before reconstitution
Store at -20 ºC after reconstitution
Avoid repeated freeze/thaw cycles of liquid mix

INTRODUCTION

Chai's Dry PCR Master Mix with Hot Start comes lyophilized for room temperature shipment. The mix contains recombinant Taq DNA polymerase, dNTPs, $MgCl_2$, KCl, cryoprotectants and bulking agents. An aptamer reversibly inhibits Taq polymerase activity at low temperatures, minimizing primer-dimers and non-specific products. The Taq is a recombinant thermostable DNA polymerase that possesses a 5'→ 3' polymerase activity and a 5'→ 3' exonuclease activity. The Master Mix amplifies products between 100 bp and 5 kb, and can be used for both PCR and qPCR (dye or probe) applications.

LIQUID 2X MASTER MIX COMPOSITION

COMPONENT	AMOUNT	FUNCTION
Taq polymerase	50 U/mL	Extends the DNA strand
Aptamer	20 nM	Inhibits Taq activity at low temp.
KCl	100 mM	Stabilizes the DNA strands
$MgCl_2$	6 mM	Co-factor for Taq polymerase
TrisCl pH 8.6	20 mM	Buffer
Glycerol	10%	Enhancer and stabilizer
Trehalose	200 mM	Enhancer
BSA	0.4 mg/mL	Enhancer and stabilizer
Detergents	0.26%	Stabilizer
dNTPs (each)	600 µM	Required for strand elongation

PROTOCOL

Reconstitution: Remove seal and cap from the vial of freeze-dried PCR Master Mix with Hot Start. Add 0.66 mL reconstitution buffer to the freeze-dried vial. Gently pipette up and down at least 10 times to ensure solution homogeneity. Avoid bubbles. Do not vortex. The liquid 2X Master Mix is now ready for use.

Reaction Set Up: Assemble the reaction components on ice (preferred) or at room temperature (up to 30 °C). The recommended reaction volume is 25 µL. Reaction volumes between 10 and 50 µL may be used; scale up/down the reaction components accordingly. Transfer the reaction(s) to a thermocycler. No separate activation step is required. Final concentration of the Master Mix in the reaction should be 1X.

COMPONENT	25 µL REACTION VOLUME	
Forward Primer	0.2 - 1 µM	
Reverse Primer	0.2 - 1 µM	
Template DNA	1 ng - 1 µg	Genomic
	0.5 pg - 5 ng	Plasmid/Viral
	1 - 100 ng	cDNA
qPCR Detection	1.25 µL	20X Chai Green
	50 - 250 nM	Probe
2X Master Mix + Hot Start	12.5 µL	
Nuclease-Free Water	Bring up to 25 µL	

THERMOCYCLING CONDITIONS

Three-Step PCR

STEP	TEMP.	TIME
Initial Denaturation	95 °C	2 min
Denature	95 °C	15 - 30 s
Anneal	45 - 68 °C	15 - 30 s
Extend	68 °C	1 min/kb
Final Extension	68 °C	5 min
Hold	4 °C	

● Cycle 30 - 40x

Two-Step PCR

When primer annealing temperatures are above 60 °C, a two-step PCR can be used:

STEP	TEMP.	TIME
Initial Denaturation	95 °C	2 min
Denature	95 °C	15 - 30 s
Anneal/Extension	60 °C	1 min/kb
Final Extension	68 °C	5 min
Hold	4 °C	

● Cycle 30 - 40x

QUALITY CONTROL ASSAY

Efficiencies of greater than 95% have been obtained using Chai's Dry PCR Master Mix with Hot Start.

Efficiency and R^2 were determined by using a five-point standard curve with lambda phage genomic DNA as template and Chai Green dye/equivalent or by using ten-fold serial dilutions of GAPDH/HPRT gene fragments with FAM and HEX labeled probes in both singleplex and duplex reactions.

Absence of endonuclease activity was determined by incubation of *E. coli* amplified DNA with 25 U Taq polymerase and monitored for little or no decrease in original amount of amplicon.

Hot Start Activity of Taq polymerase was determined by using a primer-dimer assay. Absence of primer-dimer formation in comparison to a non-Hot Start control that shows the presence of primer-dimers was used as an indicator of Hot Start activity.