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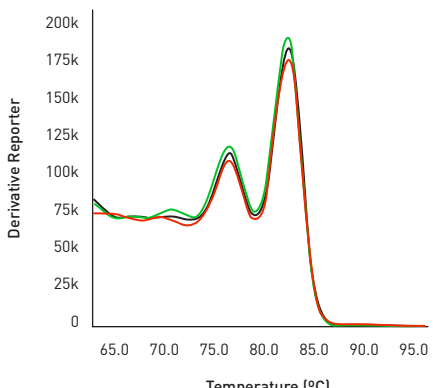
001597 Rev A

qPCR Amplification & Melt Curve Analysis with 2X Chai Green Master Mix

Two gene products, GAPDH (T_m =82.4) and HPRT (T_m=77.9) were amplified from a single gene fragment in the presence of 1X Chai Green and analyzed by Melt Curve Analysis (MCA). Two distinct peaks for the two products are obtained. The MCA was performed in triplicate.

Chai Green used for Melt Curve Analysis



QUALITY CONTROL ASSAY

Efficiency (≥95%) and R^2 (0.999) was determined using a five-point Standard Curve qPCR assay with lambda phage genomic DNA as template.

Absence of endonuclease activity was determined by overnight incubation of *E. coli* amplified DNA with 25 U Taq polymerase, in 1X reaction buffer at 37 °C, and monitored by little or no decrease in original amount of amplicon when resolved by gel electrophoresis.

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

CHAI GREEN

Master Mix + Hot Start 2X

Catalog #: R02200

For research use only

Store at -20 °C
Avoid repeated freeze-thaw
Protect from light

INTRODUCTION

2X Chai Green Master Mix + Hot Start is an optimized ready-to-use solution containing recombinant Taq DNA polymerase, an aptamer that reversibly inhibits Taq polymerase activity at low temperatures, Chai Green dye, dNTPs, $MgCl_2$, KCl and stabilizers. This inhibition prevents the formation of non-specific products and minimizes the formation of primer dimers. The Taq polymerase is a recombinant thermostable DNA polymerase that possesses a 5'→ 3' polymerase activity and a 5'→ 3' exonuclease activity. Chai Green binds to the minor groove of DNA and displays enhanced fluorescence when bound to double-stranded DNA. Its excitation-emission spectrum is very similar to SYBR Green I and hence can be used on any qPCR instrument compatible with SYBR Green. 2X Chai Green Master Mix shows decreased PCR inhibition and increased fluorescence compared to SYBR Green, and is designed for both standard melt curve analysis and HRM (High Resolution Melt).

2X CHAI GREEN MASTER MIX + HOT START 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
Chai Green	10 µM	DNA binding fluorescent dye

PROTOCOL

Thaw 2X Chai Green Master Mix + Hot Start at room temperature. Gently mix the Master Mix and spin it briefly in a microcentrifuge to collect the material in the bottom of the tube.

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 of 10 - 50 µL may be used; scale reaction components appropriately. Transfer the reaction(s) to a themocycler. No separate activation step is required. Final concentration of the Master Mix in the reaction should be 1X. For data acquisition, use the FAM/SYBR green channel. The reaction setup is shown below.

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 ng - 100 ng	cDNA
2X Master Mix	12.5 µL	
Nuclease-Free Water	Bring up to 25 µL	

THERMOCYCLING CONDITIONS

THREE-STEP PCR

For qPCR and PCR with primers having annealing temperatures ≥ 60 °C, the annealing and extension steps may be combined.

STEP	TEMP.	TIME
Initial denaturation	95 °C	30 s - 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

Chai Green Fluorescence Spectrum