



2 x SYBR[®] Green I Hot Start Real-Time PCR Mix



DESCRIPTION

2x SYBR[®] Green I Hot-Start Real-Time PCR-Mix is a 2 x-concentrated, ready-to-use reaction cocktail containing all components, except primers, for the amplification and detection of DNA in Real-Time quantitative PCR with intercalating dye SYBR[®] Green I.

Recombinant **Q-Therm[™] Taq DNA polymerase** is a variant of Taq DNA polymerase with a genetically modified *pol* gene providing a Hot-Start for PCR. The modification renders the enzyme inactive at 37°C. At temperatures over 68°C the enzyme restores its activity. Pre-heating at 95°C is not needed to activate this enzyme.

CONCENTRATION

2x

STORAGE TEMPERATURE

Store 2x SYBR[®] Green I Hot-Start real-time PCR-Mix at -15 to -25°C. For short-term storage the master mix may be stored at +2 to +8°C. Keep the master mix away from light. Avoid repeated freezing and thawing.

2 x PCR MIX COMPOSITION

Q-Therm[™] Taq DNA polymerase 0.05 units/μl

20 mM Tris-HCl pH 8.8 (at 25°C)

110 mM KCl

0.05% Tween-20

5 mM MgCl₂

dNTPs 0.4 mM of each

SYBR[®] Green I

PACK SIZE

2 x 1.25 ml (for 200 reactions in 25 μl assay)

For 25 μl Real-time PCR reaction add the following components:

	volume	final concentration
2x SYBR [®] Green I Hot-Start real-time PCR-Mix	12.5 μl	x1
forward primer	X μl*	50 – 300 nM
reverse primer	X μl*	50 – 300 nM
sample (cDNA, plasmide DNA or genomic DNA)	X μl**	20 -50 ng/reaction
H ₂ O	to 25 μl	

* volume depends on primers initial concentration. ** volume depends on template initial concentration.

Amplification protocol (for Bio-Rad iCycler):

	temperature	time	number of cycles
pre-denaturation	95°C	1 min	
denaturation	95°C	10 sec	x40
annealing	T° primers annealing *	~ 10 sec ***	
extension	72°C	~ 10 sec ****	
data collection	T° amplicon melting **	10 sec	
melt curve construction	55°C (increase setpoint temperature after each step by 0,5°)	10 sec	x80

* primers annealing temperature is selected experimentally. As the «starting point» use temperature calculated by means of appropriate program (for example AnnHyb or Oligo analyzer). ** amplification product melting temperature is defined by melting curve analysis. *** anneal time depends on primer structure and is selected experimentally. **** extension time depends on amplicon length. Preliminary as the starting point.

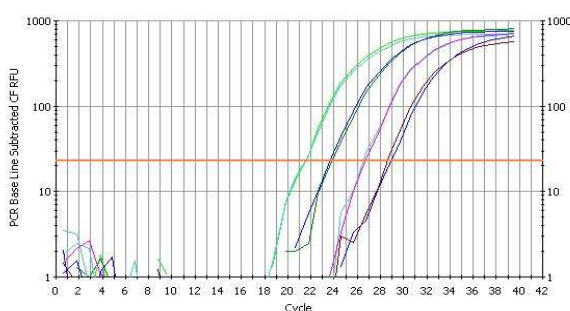
An example of the quantitative PCR with human PII gene primers and the standard curve construction:

	volume	working concentration
2x SYBR® Green I Hot-Start real-time PCR-Mix	12.5 µl	x1
forward primer (1.5 µM)	5 µl	300 nM
reverse primer (1.5 µM)	5 µl	300 nM
cDNA sample	2.5 µl	Use four point. cDNA concentration for the first point is 40 ng/reaction. cDNA concentration for the each of the subsequent point four time less than for the previous point.

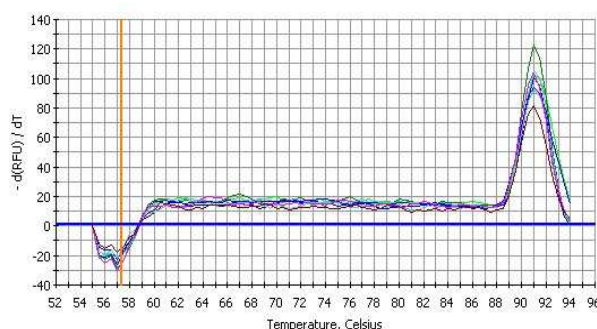
Amplification protocol (Bio-Rad iCycler):

	temperature	time	number of cycles
pre-denaturation	95°C	1 min	
denaturation	95°C	10 sec	x40
annealing	63°C	6 sec	
extension	72°C	6 sec	
data collection	88°C	10 sec	
melt curve construction	55°C (increase setpoint temperature after each step by 0,5°)	10 sec	x80

PCR amplification curve



Melting curve



standard curve

Correlation Coefficient: 0.998 Slope: -4.026 Intercept: 33.796 $Y = -4.026 X + 33.796$
 PCR Efficiency: 77.2 %

□ Unknowns
 ○ Standards

