

## Manual for 2x SYBR® Green I Hot-Start real-time PCR-Mix

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 <sub>2</sub> 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
<b>pre-denaturation</b>	95°C	1 min	
<b>Denaturation</b>	95°C	10 sec	x40
<b>Annealing</b>	T° primers annealing *	~ 10 sec ***	
<b>Extension</b>	72°C	~ 10 sec ****	
<b>data collection</b>	T° amplicon melting **	10 sec	
<b>melt curve construction</b>	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.

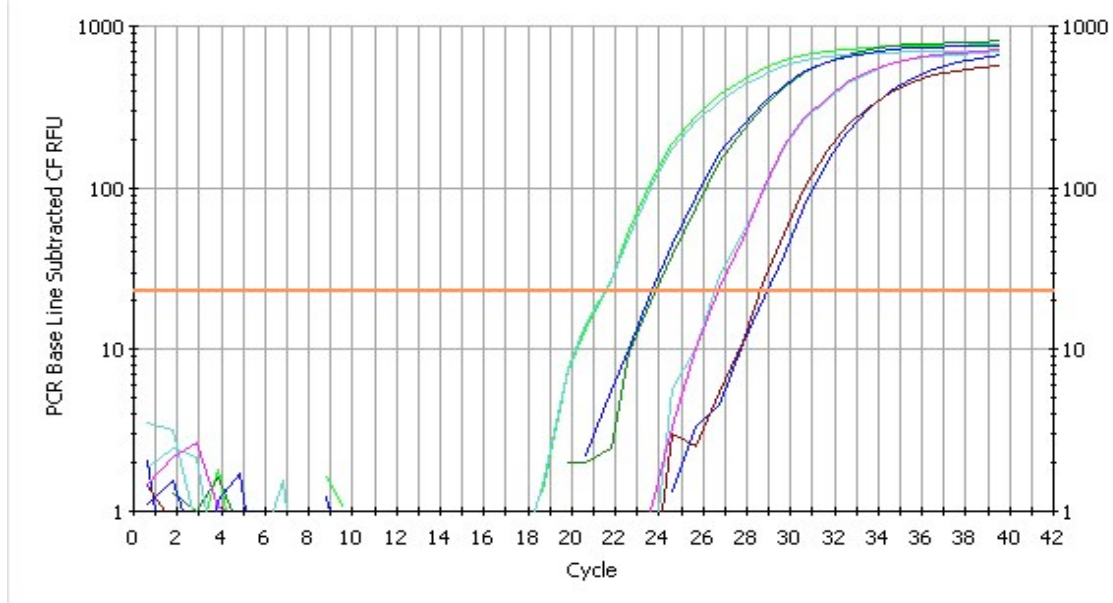
**An example of the quantitative PCR with human PII gene primers and the standard curve construction is given below:**

	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.

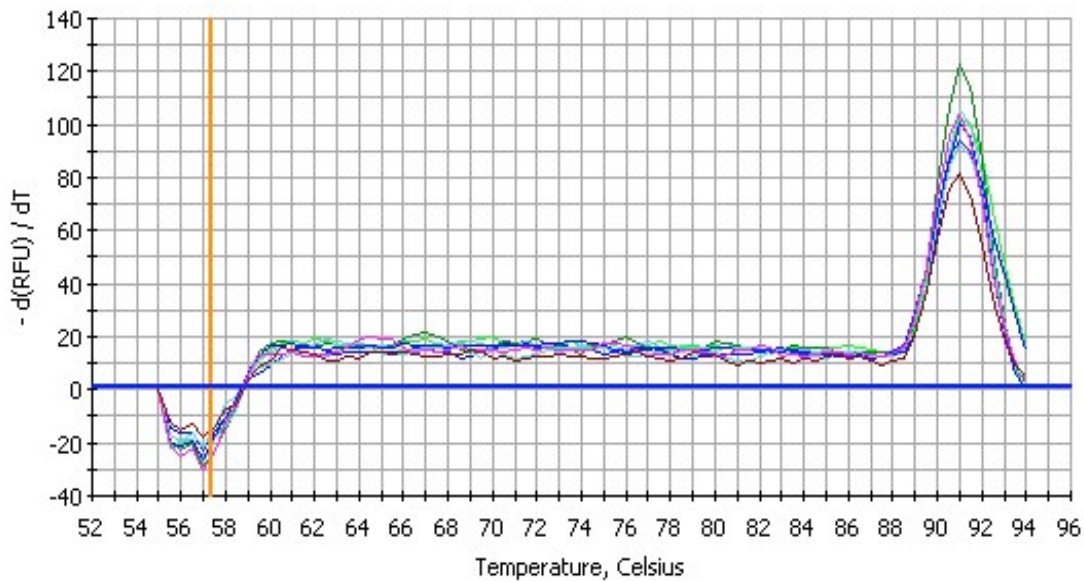
**Amplification protocol (Bio-Rad iCycler):**

	temperature	time	number of cycles
<b>pre-denaturation</b>	95°C	1 min	
<b>denaturation</b>	95°C	10 sec	x40
<b>annealing</b>	63°C	6 sec	
<b>extension</b>	72°C	6 sec	
<b>data collection</b>	88°C	10 sec	
<b>melt curve construction</b>	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

