



PROTOCOL TthPlus™ DNA Polymerase

EXPERIMENTAL PROTOCOL

Material

- HCV Control cRNA (10,000,000; 1,000,000; 100,000; 10,000; 1,000; 100; 50; 10 molecules per 8 well Control strip [8 tubes/strip or 0.1 ml tubes, respectively]).
- rTth DANN Polymerase, 2.5 u/μl, 5 x EZ-buffer, Mn-acetat-solution – 25 mM.
- TthPlus DANN Polymerase, 5 u/μl; 10 x one-tube RT-PCR-buffer; Mn-acetate-solution – 50 mM

Instruments

- ABI PRISM 7000 Sequence Detection System
- Rotor-Gene 2000

REACTIONS AND CONDITIONS (BOTH INSTRUMENTS)

Reaction component	Final concentration (25 μl-Assay)
10x/5x buffer	1x
Mn-acetate-solution	3.5 mM
Nucleotide mix	0.3 mM dATP, dCTP and dGTP, 0.6 mM dUTP
forward and reverse primer	7.5 pmol each
TaqMan probe	3.4 pmol
Tth DNA Polymerase	1.5 u

CYCLER PROGRAM

Rotor-Gene	RT	Hold	Cycle		Hold
Temperature (°C)	59	95	95	59	25
Time (mins)					
Cycles	60	10:00	00:15 45	01:00	∞

shut off „9600 emulation“ (window „instrument“)

7000 SDS	RT	Hold	Cycle		Hold
Temperature (°C)	59	95	96	59	25
Time (mins)					
Cycles	60	10:00	00:30 40	01:30	∞