



TaqMan real-time PCR for the detection of SARS Coronavirus

PCR system:

Quantitative real-time PCR for the detection of SARS Coronavirus RNA (1-step RT-PCR) or cDNA

Real-time PCR concept:

5' Nuclease Oligoprobe (TaqMan)

Assay concept:

The assay detects 3 genes that are distributed over the SARS Coronavirus genome either in single reactions or in a combined reaction of the 3 single assays.

Applied oligonucleotides

Table 1:

Name	Primer/probe sequence ^a		Position ^c	Tm ^d
NS pp1a (133 bp)^b				
pp1a F	GCCgTAGTgTCAgTATCATCACC	S	4609-4631	56.6°C
pp1a R	AATAggACCAATCTCTgTAAgAgCC	A	4741-4717	56.7°C
pp1a TM	F -TCACTTCgTCATCAAAGACATC X <u>T</u> gAggAgC p	S	4661-4690	66.2°C
NS pp1ab (88 bp)^b				
NS F	TTTTgTTgTTTCAACTggATACCAT	S	14387-411	57.0°C
NS R	GAAACTgAgACgCgAgCTATgT	A	14474-453	57.3°C
NS TM	F -CATCCTgATTATgTACgACTCCTAAC X <u>T</u> CACgAA p	A	14445-413	64.4°C
Surface spike glycoprotein (79 bp)^b				
SS GP F	gAggTCTTTTATTgAggACTTgCTC	S	23879-903	57.1°C
SS GP R	gCATTCgCCATATTgCTTCAT	A	23957-937	57.3°C
SS GP TM	F -AAgCCAgCATCAgCgAgTgTCACCTTA X <u>T</u> p	A	23935-908	66.7°C

^a in 5'→3' direction/ S Sense orientation, A Antisense orientation/ ^b Amplicon size in base pairs/ ^c Position in Genbank entry AY274119 isolate TOR2/ ^d Thermodynamic melting temperature/ **F**:FAM / **X**: TAMRA / **p**: phosphate. A SARS coronavirus standard preparation can be obtained at the Robert Koch-Institut, Berlin, Germany, Dr. M. Niedrig: Phone +49-1888-754-2370, Fax +49-1888-754-2625; and <http://www.rki.de/INFEKT/SARS/DATASHEET.PDF>.

Reaction platforms

This assay has been tested on the following real-time PCR machines:

Applied Biosystems (Foster City, CA, USA)
 SDS 7000
 SDS 7700
 SDS 7900HT

LightCycler (Roche Diagnostics, Mannheim, Germany)

Table 2: Reaction conditions for the SARS Coronavirus-specific 1-step RT-PCR in 96-well format as used for the Applied Biosystems real-time PCR platforms

	single	combination	temperature	time	cycles
2xQuantiTect Probe RT Master Mix*	12.5 µL	12.5 µL	50°C	30 min	1x
QuantiTect Probe RT Mix*	0.25 µL	0.25 µL	95°C	15 min	1x
Primer F [§]	10 pmol	3 x 10 pmol	95°C	15 s	45x
Primer R [§]	10 pmol	3 x 10 pmol	60°C	30 s	
Nuclease probe TM [§]	3 pmol	3 x 3 pmol			
RNAse free PCR water	ad 23.0 µL	ad 23.0 µL			
Template RNA	2 µL	2 µL			
Reaction volume	25 µL	25 µL			

*Qiagen, Hilden, Germany; [§] see table 1, all primers are used for the combined assay

Table 3: Reaction conditions for the SARS Coronavirus-specific 2-step RT-PCR in 96-well format as used for the Applied Biosystems real-time PCR platforms

	single	combination	temperature	time	cycles
10x Real-time PCR Mix*	2.5 µL	2.5 µL	50°C	30 min	1x
Primer F [§]	10 pmol	3 x 10 pmol	95°C	15 min	1x
Primer R [§]	10 pmol	3 x 10 pmol	95°C	15 s	45x
Nuclease probe TM [§]	3 pmol	3 x 3 pmol	60°C	30 s	
PCR water	ad 23.0 µL	ad 23.0 µL			
Template cDNA	2 µL	2 µL			
Reaction volume	25 µL	25 µL			

*Tested for PCR master mixes provided by ABGene, Applied Biosystems and Invitrogen; [§]see table 1, all primers are used for the combined assay

Table 4: Reaction conditions for the SARS Coronavirus-specific 1-step RT-PCR in glass capillaries as used for the Roche LightCycler

	single	combination	temperature	time	cycles
2.7x Mix LC Master RNA Mix*	7.5 µL	7.5 µL	55°C	20 min	1x
Mn(OAc) ₂	3.25 mM	3.25 mM	95°C	30 s	1x
primer F [§]	10 pmol	3 x 10 pmol	95°C	1 s	45x
primer R [§]	10 pmol	3 x 10 pmol	55°C	10 s	
Nuclease probe TM	3 pmol	3 x 3 pmol	72°C	10 s	
RNAse free PCR water	ad 18 µL	ad 18 µL	30°C	30 s	
Template RNA	2.0 µL	2.0 µL			
Reaction volume	20 µL	20 µL			

* Roche Applied Science; [§]see table 1, all primers are used for the combined assay

Table 5: Reaction conditions for the SARS Coronavirus-specific 2-step RT-PCR in glass capillaries as used for the Roche LightCycler

	single	combination	temperature	time	cycles
FastStart Master Mix*	2.0 µL	2.0 µL	50°C	120 s	1x
MgCl ₂	5.0 mM	5.0 mM	95°C	30 s	1x
primer F [§]	10 pmol	3 x 10 pmol	95°C	1 s	45x
primer R [§]	10 pmol	3 x 10 pmol	55°C	10 s	
Nuclease probe TM	3 pmol	3 x 3 pmol	72°C	10 s	
PCR water	ad 18 µL	ad 18 µL	30°C	30 s	
Template cDNA	2.0 µL	2.0 µL			
Reaction volume	20 µL	20 µL			

* Roche Applied Science; [§]see table 1, all primers are used for the combined assay