LightCycler® 480 Instrument Basic Real-time Chemistry Protocols Quick Reference Card

Reagents and Plates

Format	Name	Catalog Number	Description
Reverse Transcription with RNAse H activity, **Recommended**	Transcriptor 1st Strand cDNA Synthesis Kit	04 379 012 001	For 50 synthesis reactions
SYBR Green	LightCycler® 480 SYBR Green I Master	04 707 516 001	2 x Master Mix for 250 20 µL reactions (5 mL)
Hybridization Probes	LightCycler® 480 Genotyping Master Mix	04 707 524 001	5 x Master Mix for 250 20 µL reactions (5 mL)
TaqMan® Probes or Universal Probe Library (UPL)	LightCycler® 480 Probes Master	04707 494 001	2 x Master Mix for 250 20 µL reactions (5 mL)
96 wells	LightCycler® 480 Multiwell Plate 96	04 729 692 001	50 plates and sealing foils
384 wells	LightCycler® 480 Multiwell Plate 384	04 729 749 001	50 plates and sealing foils

Typical SYBR Green Reaction Protocol

To erase data entry steps, apply the appropriate default template from the Roche User's Templates folder.

Program name: Pre-incubation (1 cycle)
Analysis mode: none

Step One:	Pre-incubation	Target Temperature 95 °C	Hold time 5-10 minutes

Program name: Amplification (45 cycles)
Analysis mode: Quantification

Step One:	Denaturation	Target Temperature 95 °C	Hold time 10 seconds
Step Two:	Annealing	Target Temperature primer specific[1]	Hold time 10 seconds
Step Three:	Extension	Target Temperature 72 °C	Hold time[2] 10 seconds

Note: *Single fluorescence reading in step 3.*

Program name: Melting curve (1 cycle)
Analysis mode: Melting curve

Step One:	Initial hold	Target Temperature 95 °C	Hold time 5 seconds
Step Two:	Second hold	Target Temperature 65 °C	Hold time 60 seconds
Step Three:	Gradient	Target Temperature 97 °C	Continuous data acquisition; no hold

Note: *Acquire fluorescence continuously with two to five acquisitons per degree.*

[1] set annealing temperature approximately 1 to 5 degrees below the Tm of the Primers.
[2] extension time is calculated allowing 1 second per 25 base pairs of estimated amplicon length. In some cases, it may be advantageous to use longer hold times; which, could allow greater precision of target and amplification.

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Typical SYBR Green Reaction Protocol, continued

SYBR Green Setup and Programming

SYBR Wet Setup		
Reagent	**Final Concentration**	**Volume (µL)/one reaction**
Water		1.0
Primer - Forward (5 µM Stock)	0.5 µM	2.0
Primer - Reverse (5 µM Stock)	0.5 µM	2.0
LightCycler® 480 SYBR Green I Master	1X	10
TOTAL		15
Add 5 µL cDNA for a total volume of 20 µL		

SYBR Green Run Program		
Step	**Temperature (°C)**	**Time**
Initital Denaturation	95	00:05:00 (5 minutes)
Amplification	95	00:00:10 (10 seconds)
Annealing Temperature is Primer Specific	60	00:00:10 (10 seconds)
Time is Product Length/25	72	00:00:10 (10 seconds)
Melting Curve	95 65 97	00:00:05 (5 seconds) 00:01:00 (60 seconds) 00:00:00 (no hold)
Cooling	40	00:00:30 (30 seconds)

Typical Hybridization (HybProbe) Probe Reaction Protocol

To erase data entry steps, apply the appropriate default template from the Roche User's Templates folder.

Program name: Pre-incubation (1 cycle)
Analysis mode: none

Step One:	Pre-incubation	Target Temperature 95 °C	Hold time 5-10 minutes

Program name: Amplification (45 cycles)
Analysis mode: Quantification

Step One:	Denaturation	Target Temperature 95 °C	Hold time 5-10 seconds
Step Two:	Annealing	Target Temperature primer specific[3]	Hold time 5-15 seconds

Note: *Single fluorescence reading in step 2.*

Step Three:	Extension	Target Temperature 72 °C	Hold time 10-30 seconds[4]

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Typical Hybridization (HybProbe) Probe Reaction Protocol, continued

Program name: Melting Curve (1 cycle)
Analysis mode: Melting Curve

Step One:	Initial Hold	Target Temperature 95 °C	Hold time 1 minute
Step Two:	Second Hold	Target Temperature 40 °C	Hold time 2 minutes
Step Three:	Gradient	Target Temperature 95 °C	Continuous data acquisition, no hold

Note: *Acquire fluorescence continuously with 2 to 5 acqusitions per degree.*

[3] set annealing temperature approximately 1 to 5 degrees below the Tm of the Primers.
[4] extension time is calculated allowing 1 second per 25 base pairs of estimated amplicon length. In some cases, it may be advantageous to use longer hold times; which, could allow greater precision of target and amplification. Add approximately 15 seconds.

Hybridization Probe Reaction Setup and Programming

HybrProbe Wet Setup		
Reagent	**Final Concentration**	**Volume (µL)/one reaction**
Water		6.2
Primer - Forward (5 µM Stock)	0.5 µM	2.0
Primer - Reverse (5 µM Stock)	0.5 µM	2.0
Fluorescein Probe (10 µM Stock)	0.1-.05 µM	0.4
Red Fluor Probe (10 µM Stock)	0.2-0.5 µM	0.4
LightCycler® 480 Genotyping Master	1X	4.0
TOTAL		15
Add 5 µL cDNA for a total volume of 20 µL		

HybProbe Run Program		
Step	**Temperature (°C)**	**Time**
Initital Denaturation	95	00:10:00 (10 minutes)
Amplification	95	00:00:10 (10 seconds)
Annealing Temperature is Primer Specific	60	00:00:10 (10 seconds)
Time is Product Length/25	72	00:00:10 (10 seconds)
Melting Curve	95 40 95	00:01:00 (60 seconds) 00:02:00 (120 seconds) 00:00:00 (no hold)
Cooling	40	00:00:30 (30 seconds)

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Typical Hydrolysis (TaqMan®) Probe Reaction Protocol

To erase data entry steps, apply the appropriate default template from the Roche User's Templates folder.

Program name: Pre-incubation (1 cycle)
Analysis mode: none

Step One:	Pre-incubation	Target Temperature 95 °C	Hold time 5-10 minutes

Program name: Amplification (45 cycles)
Analysis mode: Quantification

Step One:	Denaturation	Target Temperature 95 °C	Hold time 10 seconds
Step Two:	Amplification and	Target Temperature 60 °C	Hold time 20-60 seconds
Step Three:	Extension	Target Temperature 72 °C	Hold time 1 second

Note: *Single fluorescence reading in step 2.*

TaqMan® Probe Setup and Programming

Universal Probe or TaqMan® Probe Setup		
Reagent	**Final Concentration**	**Volume (µL)/one reaction**
Water		0.6
Primer - Forward (5 µM Stock)	0.5 µM	2.0
Primer - Reverse (5 µM Stock)	0.5 µM	2.0
Probe (10 µM Stock)	0.1 - 0.2 µM	0.4
LightCycler® 480 Probe Master	1X	10
TOTAL		15
Add 5 µL cDNA for a total volume of 20 µL		

SYBR Green Run Program		
Step	**Temperature (°C)**	**Time**
Initital Denaturation	95	00:10:00 (10 minutes)
Amplification	95 60	00:00:10 (10 seconds) 00:00:30 (10 seconds)
Extension	72	00:00:01 (1 second)
Cooling	40	00:00:30 (30 seconds)

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