

LightCycler[®] 480 Instrument Quick Guide

Relative Quantification

Performing a Relative Quantification Analysis:

- 1. Open the experiment that you want to analyze in main window¹.
- 2. If sample information was entered during run setup then proceed to <u>Step 5</u>; otherwise click the <**Sample Editor**> object on the left of the active display.
- 3. In the **<Sample Editor**> select the Workflow **<Rel Quant**> under Step 1.



¹ If the experiment has just completed, the run will remain open and ready to analyze. Previously created and performed experiments will be located in the *Experiments* Folder of the Navigator.

- 4. To enter the information for like samples:
 - a. Select control samples on the plate layout (these are the baseline or untreated samples).

Window:	New Experiment
Experi- ment	Step 1: Select Workflow C Abs Quant
Subset Editor	Step 2: Select Samples
Sample Editor	
Analysis	
Report	

b. Enter sample name and choose Sample Type **<Positive Control Calibrator**>.

Window:	New Experiment
Experi- ment	Cstep 1: Select Workflow ○ Abs Quant
Subset Editor Sample Editor	Subset 111 Samples 1 2 3 4 5 6 7 8 9 10 11 12 A 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Report	Step 3: Edit Rel Quant Properties
	Simple Type Unknown Negative Control Positive Control/Calibrator Standard Concentration Auto Std Curve Gene target
	Target name Eff 2.00 C Target C Reference © Unassigned Make Replicates
	Apply Template Configure Toggle View Properties (Table)

c. Enter target name (e.g., name of the gene being amplified in the selected wells), and select gene target information related to the relative quantification analysis (identity gene target as "Target" or "Reference." "Target" is the **gene of interest** and "Reference" is the **Housekeeping Gene** (e.g., actin).

Sum.	Step 3: Edit Rel Quant Properties Sample Name Sample Type © Unknown O Negative Control Positive Control/Calibrator	
	C Standard Concentration Auto Std Curve Gene target Target name Eff 2.00 Target C Reference Unassigned	
	Make Replicates	
	Apply ✔ Configure Configure (Toggle View Properties (Table)	

- d. For all other samples, enter sample name and choose Sample Type <Unknown or Negative Control> (refer to step f for setting up Standards).
- e. Enter gene name and define Target or Reference as described in step c.
- f. Click on wells with one standard concentration and enter sample names, concentration and click **Standard**. Repeat for other concentrations being used.

step 5. Luit Kei Quan	triopenies
Sample Name	
-Sample Type	
O Unknown	C Negative Control
<u>O Positive Control/C</u>	alibrator
Standard Concer	Auto Std Curve

g. Set replicates by clicking Auto Replicate.

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ampre Name j	
Sample Type-	228
📀 Unknown	🔿 Negative Control
Positive Con	ntrol/Calibrator
C Standard C	oncentration Auto Std Curve
Gene target—	
Gene target— Target name	Eff 2.00
Gene target— Target name 🗍 🔿 Target	Eff 2.00 C Reference
Gene target Target name 🗍 🔿 Target	Eff 2.00 C Reference (• Unassigned
Gene target Target name 🗍 (^ Target	Eff 2.00 C Reference (• Unassigned
Gene target Target name Target Target	Eff 2.00 Reference Make Replicates

- 5. Click <**Analysis**>.
 - a. Select Advanced Relative Quantification.



b. Select all samples or subset to be analyzed, and click the checkmark icon.

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c. Select default settings and click the checkmark icon.



d. The software automatically matches the target to the corresponding reference.

	Results			Manual Pairin	g		Target Name		
Bar			Tar	get Name	Target	Reference	Rat	ios	
Chart	Pairing	Sample Name	Targets	References	Mean Cp	Mean Cp	Target/Ref	Normalized	Status
		Calibrator	Target 1	Reference 1; Refere					
~	A1/D1	sample 1	Target 1	Reference 1;Refere					
~	A2/D2	sample 2	Target 1	Reference 1;Refere					
~	A3/D3	sample 3	Target 1	Reference 1; Refere					
~	A4/D4	sample 4	Target 1	Reference 1;Refere					
~	AS/DS	sample S	Target 1	Reference 1; Refere					
~		calibrator	Target 2	Reference 1;Refere					
		Sample V	iew				Bar Char		
				Relative Quantifi	ation Resul	ts			
ų				Novembr	o display				
Ratio				No results	to display.				

- e. To obtain results, click <**Calculate**>.
 - If a calibrator sample was used, the column of interest is the normalized ratio which gives you the efficiency corrected normalized fold change of the sample over the calibrator control. A number of 2 in this column would mean a two-fold increase compared to the calibrator sample as the normal ratio.
 - If a calibrator sample was not used, then the Target/Ref Ratio column will give your results, comparing target to reference amounts within a single sample.

Experi-	Analyses Advanced Relative Quantification for All Samples (1)										┘ぬ⊕⊝⊘
ment	Informat	ion Subset:	All Samples, Pro	gram: amplificati	on, References: In-R	un, Abs Q	ant Type:	Abs Quant/2nd	l Derivative	Hax	
Subset			Results		Manual Pairin	g			Target Nan	10	
Eattor	Bar			Targ	et Name	Target	Reference	Rati	ios		-
Samula	Chart	Pairing	Sample Name	Targets	References	Mean Cp	Mean Cp	Target/Ref	Normalized	Status	
Editor	~		calibrator	Target 1	Reference 1;Refere	22.43	24.14	3.266	1.000		
\square	~	A1/D1	sample 1	Target 1	Reference 1;Refere	23.76	26.31	5.859	1.794		
	~	A2/D2	sample 2	Target 1	Reference 1;Refere	21.34	24.58	9,490	2.906		
Analysis	v	A3/D3	sample 3	Target 1	Reference 1;Refere	24.30	26.18	3.675	1.125		
	•	A4/D4	sample 4	Target 1	Reference 1;Refere	23.56	25.27	3.267	1.000		
	•	A5/D5	sample 5	Target 1	Reference 1;Refere	25.11	23.90	0.4320	0.1323		
Report			calibrator	Target 2	Reference 1;Refere	28.49	24.14	4.91E-2	1.000		•
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Sum.			Sample	View				Bar Chart			
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	Ratio	W									
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Note: All results are exportable to other programs. Export the graphics or data table by right-clicking the object and follow the export windows.

To export the Data, select the data by clicking on a row. Click < **Ctrl**> and <**A**> together to select all, click < **Ctrl**> and <**C**> together to copy and < **Ctrl**> and <**V**> together to paste into another presentation, spreadsheet or document.

f. Click the **Target Name** tab, and double-click on the corresponding **Target Name** to view, edit settings, or remove samples from the analysis.

🗗 LightCy	rcler® 480 Software re	lease 1.5.0 SP3					
Instrumen	t: Virtual LightCycle	er 480 96 System II / Not Conne	cted		1	Database:	My Computer (Research)
Window:	Demo Rel Quant	Mono Color	•	User:	System Admin		
Experi- ment	Analyses Advanced	N Relative Quantification	for All Samples (1)				7 800X
\square	Information Subset	: All Samples, Program:	amplification, Reference	es: In-Run, Abs Quant	Type: Abs	Quant/21	ud Derivative Max
Subset		Results	Mar	Manual Pairing			Target Name
Editor	Target Name	Filter Combination	Standards/Efficiency	Efficiency Value			
	Target 1	465-510	Efficiency	2.00			
Sample	Target 2	465-510	Efficiency	2.00			
Laitor	Reference 1	465-510	Efficiency	2.00			
\equiv	Reference 2	465-510	Efficiency	2.00			
Analysis							

g. Once sample editing is done, click calculate, and then click **Back to Rel Quant** icon.



h. Click the Rel Quant **Results** tab, and then click **Calculate** to obtain updated results.

Experi-	Analyses Advanced Relative Quantification for All Samples (1)] ∰⊕⊝[⁄
ment	Informat	ion Subset	: All Samples, P:	cogram: amplification	n, References: In-R	un, Abs Qua	ant Type:	Abs Quant/2n	d Derivative	Max	
Subset	[Results		Manual Pairin	g			Target Nam	ie	
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Samula	Chart	Pairing	Sample Name	Targets	References	Mean Cp	Mean Cp	Target/Ref	Normalized	Status	
Editor	~		calibrator	Target 1	Reference 1;Refere	22.43	24.14	3.266	1.000		
	•	A1/D1	sample 1	Target 1	Reference 1;Refere	23.76	26.31	5.859	1.794		
	✓	A2/D2	sample 2	Target 1	Reference 1;Refere	21.34	24.58	9.490	2.906		
Analysis	v	A3/D3	sample 3	Target 1	Reference 1;Refere	24.30	26.18	3.675	1.125		
	~	£4/D4	sample 4	Target 1	Reference 1;Refere	23.56	25.27	3.267	1.000		
	~	AS/DS	sample 5	Target 1	Reference 1;Refere	25.11	23.90	0.4320	0.1323		
Report	~		calibrator	Target 2	Reference 1;Refere	28.49	24.14	4.91E-2	1.000		-
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LightCycler[®] 480 Reagents and Disposables:

Product name	Cat. No.	Pack Size/Description
LightCycler [®] 480 SYBR Green I Master	04 707 516 001	5mL (5 x 1mL)
LightCycler [®] 480 Probes Master	04 707 494 001	5mL (5 x 1mL)
LightCycler [®] 480 Genotyping Master	04 707 524 001	Master Mix, 5x conc., 4 x 384 μ l, ready-to-use hot start multiplex PCR reaction mix, containing a modified Taq DNA polymerase, reaction buffer, dNTP mix (with UTP instead of dTTP) and 15 mM MgCl ₂
LightCycler [®] 480 Control Kit	04 710 924 001	Kit for quantitative real-time PCR and genotyping control reactions using the LightCycler [®] 480 Instrument

Product name	Cat. No.	Pack Size/ Description
LightCycler [®] 480 Multiwell Plate 96	04 729 692 001	$5 \ge 10$ plates and sealing foils
LightCycler [®] 480 Multiwell Plate 384	04 729 749 001	$5 \ge 10$ plates and sealing foils
LightCycler [®] 480 Sealing Foil	04 729 757 001	50 foils

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