LightCycler 2.0
DNA R-gene® Kits
Programming

- **The use of these guideline is dedicated for Argene kits that mentionned the instrument LightCycler 2.0 in their package insert.**

- **Some products have their own amplification program following the same principle. In this case, please refer to their Outlined procedure.**

Products to be amplified relate to the extracted DNA obtained with the extraction methods recommended in the corresponding datasheet.

Plan the experiment as described in the datasheet.

- **AMPLIFICATION PROGRAM: "60°C R-gene" Program**

Steps		Time	Temperature	Cycles	Wavelength for signal reading
Taq Polymerase Activation		15 min.	95°C	1	-
Amplification	Denaturation	10 sec	95°C	45	-
	Annealing Elongation	40 sec.	60°C		530 and/or 560 nm end of the annealing
Cooling (*Optional*)		30 sec.	40°C	1	-

Note: Temperature transition rate/slope is predefined at 20°C/sec.

1. Throughout the patient follow-up, it is imperative to use the same protocol and to use the same extraction and amplification instrument.

2. This guideline is based on the LightCycler2 Version 4.05 Software.

LightCycler 2.0 Programming

In the room reserved for amplification

For multiple wavelength detection, the use of a colour compensation file is imperative to interpret the results on the LightCycler 2.0

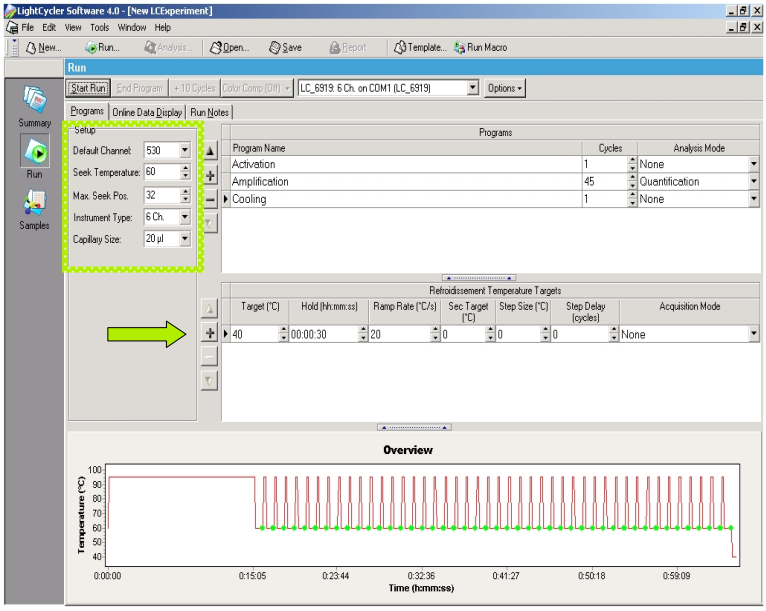
Check that this file was created using the Colour Compensation r-gene® (Argene, ref.: 71-103) in the LightCycler 2.0 software.

STARTING THE LIGHTCYCLER SOFTWARE VERSION 4.05

- Switch on the computer, its screen, the LightCycler 2.0 and the LC Carrousel Centrifuge.
- Start the LightCycler Software by clicking its icon LIGHTCYCLER SOFTWARE VERSION 4.
- Enter the user name and the software password.
- Click on RUN to open the programming and run module.

ENTRY OF THE PROGRAM DATA

- Set the SEEK TEMPERATURE to 60.
- Set the CAPILLARY SIZE to 20 µL.
- Set the DEFAULT CHANNEL to 530.



ENTRY OF THE AMPLIFICATION PARAMETERS

- In PROGRAM NAME click the «+» 2 times.
  - Enter ACTIVATION in the 1st row.
  - Enter AMPLIFICATION in the 2nd row.
  - Enter COOLING in the 3rd row.
- In the CYCLES section:
  - Enter 1 in the 1st row.
  - Enter 45* in the 2nd row.
    * Number of cycles for Amplification
  - Enter 1 in the 3rd row.
- In the ANALYSIS MODE section:
  - Select NONE in the 1st row.
  - Select QUANTIFICATION in the 2nd row.
  - Select NONE in the 3rd row.

Hot Start Taq Activation

- Click on the ACTIVATION program.
  - Set 95 in the TARGET field.
  - Set 00:15:00 in the HOLD field.

Denaturation step

- Click on the AMPLIFICATION program, and click the «+» 1 time.
  - Set 95 in the 1st row of the TARGET field.
  - Set 00:00:10 in the 1st row of the HOLD field.

Annealing / Elongation step

- Set 60 in the 2nd row of the TARGET field.
- Set 00:00:40 in the 2nd row of the HOLD field.
- Select SINGLE in the ACQUISITION MODE field.

- Click on the COOLING program.
  - Set 40 in the TARGET field.
  - Set 00:00:30 in the HOLD field.

Note: **If this program has already been saved, it can be launched directly by clicking on RUN MACRO and selecting the appropriate program (FAM RGENE).**

- **ENTRY OF PARAMETERS FOR RESULTS INTERPRETATION**
  - In the SAMPLE tab, click on the ANALYSIS TYPE tab.
  - Select ABSOLUTE QUANTIFICATION.
  - Select 530 nm and/or 560 nm channel(s).
    m Use the 560 nm channel only for multiple wavelength detection
  - In the UNITS field, enter COPIES/ML.
- **SAVE THE "60°C RGENE" PROGRAM**
  - Click on TOOLS to store this program for subsequent experiments.
  - Click on CREATE MACRO/KIT TEMPLATE**.**
  - Select EXPERIMENT KIT MACRO then click OK.
  - Name the program: 60°C R-GENE and save it in the folder TEMPLATES AND MACROS.

Running the Program

- Run the amplification program (stored according to the instructions described in section "LightCycler 2.0 Programming") by clicking on RUN in the START RUN tab.
- Type the name of your file *(*ex.: EBV RGENE YYYY-MM-DD*)* and store the file.

- **DEFINE THE SAMPLE**
  - Click on SAMPLES to specify the samples, standards and controls to be amplified in each position of the carrousel.
  - Fill the SAMPLE NAME column in the CAPILLARY VIEW tab.
  - Define the samples in the ABS QUANT tab as described in the table below.

	Quantitative kits		Qualitative kits	
Channels	530	560	530	560
Patient samples	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN
Quantification standard (**QS**)	STANDARD	UNKNOWN	Not applicable	
Sensitivity control (**SC**)	UNKNOWN	UNKNOWN	Not applicable	
Extraction+inhibition control (**IC2W0**)	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN
Positive control (**PC**)	Not applicable		UNKNOWN	UNKNOWN
Negative Amplification Control (**R0**)	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN

If several parameters are detected in the same experiment, each quantification standard range has to be identified one by one. Not applicable for qualitative detection kits.

ONLY FOR QUANTIFICATION:

- Enter the concentration of the standard(s) (cp/mL) in the **CONCENTRATION** column.

Conditions					Standard concentration to be entered			
R-gene® Kit	**Extraction method**	**Specimen**	**Sample volume to be extracted**	**Elution volume**	**QS1**	**QS2**	**QS3**	**QS4**
69-002 EBV R-gene®	QIAamp DNA Blood Mini kit	Whole blood	200 µL	100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	QIAcube	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure Compact MagNA Pure LC System	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	NucliSENS® easyMAG®	Whole blood / Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	BioRobot EZ1 Workstation	Whole blood	350 µL	200 µL	6 000 000	600 000	60 000	6 000
	m2000sp Abbott	Whole blood / plasma / CSF / BAL / biopsies	800 µL (extract 300 µL)	250 µL (eluate 150 µL)	8 000 000	800 000	80 000	8 000
	Versant kPCR Molecular System SP	Plasma	400 µL (extract 250 µL)	65 µL (eluate 50µL)	2 500 000	250 000	25 000	2 500
69-003 CMV R-gene® **69-100** CMV HHV6, 7,8 R-gene®	QIAamp DNA Blood Mini kit	Whole blood Amniotic Fluid	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	QIAcube	Whole blood Amniotic Fluid		100 µL	2 500 000	250 000	25 000	2 500
		Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure Compact MagNA Pure LC System	Whole blood Amniotic Fluid		100 µL	2 500 000	250 000	25 000	2 500
		Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	NucliSENS® easyMAG®	Whole blood						
		Serum / Plasma / CSF						
	m2000sp Abbott	Whole blood / plasma / BAL / urine / biopsies / amniotic fluid	800 µL (extract 300 µL)	250 µL (eluate 150µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP	Plasma	400 µL (extract 250 µL)	65 µL (eluate 50µL)	1 250 000	125 000	12 500	1 250

Conditions					Standard concentration to be entered			
R-gene® Kit	Extraction method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
69-004 HSV1 HSV2 VZV R-gene® **71-015** HSV1 r-gene® **71-016** HSV2 r-gene® **71-017** VZV r-gene®	QIAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma	200 µL	50 µL	500 000	50 000	5 000	500
	QIAamp MinElute Virus Spin Kit	CSF						
	QIAcube + QIAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma						
	QIAcube + QIAamp MinElute Virus Spin Kit	CSF						
	MagNA Pure Compact MagNA Pure LC System	CSF						
	NucliSENS® easy MAG®	CSF						
	Versant kPCR Molecular System SP	CSF	400 µL (extract 250 µL)	65 µL (eluate 50µL)				
69-010 ADENOVIRUS R-gene®	QIAamp DNA Blood Mini kit	Whole blood / Respiratory samples* / Stool* / biopsies	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	QIAamp DNA Stool Mini kit	Stool*		100 µL	2 500 000	250 000	25 000	2 500
	QIAcube + QIAamp DNA Blood Mini kit	Whole blood / Respiratory samples* / Stool* / biopsies		100 µL	2 500 000	250 000	25 000	2 500
		Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	QIAamp DNA Stool Mini kit	Stool*		100 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure Compact MagNA Pure LC System	Plasma / Respiratory samples*		50 µL	1 250 000	125 000	12 500	1 250
		Whole blood / Stool*		100 µL	2 500 000	250 000	25 000	2 500
	NucliSENS® easyMAG®	Respiratory samples* / Whole blood / Stool*		50 µL	1 250 000	125 000	12 500	1 250
	BioRobot M48 QIAGEN	Respiratory samples*		100 µL	2 500 000	250 000	25 000	2 500
	m2000sp Abbott	Whole blood / biopsies / Respiratory samples*	800 µL (extract 300 µL)	250 µL (eluate 150µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP	Plasma / CSF	400 µL (extract 250 µL)	65 µL (eluate 50µL)	1 250 000	125 000	12 500	1 250

** For a quantitative detection in cp/PCR, see 69-010 datasheet, section 11.3.*

Conditions					Standard concentration to be entered			
R-gene® Kit	Extraction method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
69-013 BK Virus R-gene®	QIAamp DNA Blood Mini kit	Whole blood / Urine	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	QIAcube	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure Compact	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure LC System	Whole blood		100 µL	2 500 000	250 000	25 000	2 500
		Plasma / Urine		50 µL	1 250 000	125 000	12 500	1 250
	NucliSENS® easyMAG®	Whole blood / Plasma		50 µL	1 250 000	125 000	12 500	1 250
		Urine		100 µL	2 500 000	250 000	25 000	2 500
	Versant kPCR Molecular System SP	Plasma / Urine	400 µL (extract 250 µL)	65 µL (eluate 50µL)	1 250 000	125 000	12 500	1 250

If several parameters are detected in the same experiment, each quantification standard range has to be identified one by one. Not applicable for qualitative detection kits.

Data Analysis

GENERATE ANALYSIS FILE(S)

- At the end of the run, click **Analysis**, then **Absolute Quantification** and then **OK**.
- Click on the **Channel** tab and select the wavelength: **530nm**.
  This file enables the amplification of the targeted DNA to be followed.

Only for multiple wavelength detection:

Click **Analysis** again, then **Absolute Quantification**, then **OK**.
Click on the **Channel** tab and select the wavelength: **560nm**.
This file enables the amplification of each inhibition control related to each tested sample (after activation of the compensation colour file/object) to be followed.

ACTIVATION OF COLOUR COMPENSATION FILE/OBJECT

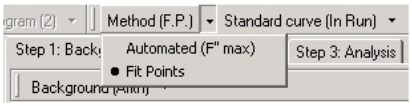
- Click the **colour compensation** tab then select the suitable file previously created with Colour Compensation r-gene® (Argene ref.: 71-103).

ANALYSIS OF SAMPLES AND STANDARDS

"Fit points" method: Identification of positive samples:

This method is used to interpret the signal read at 530 nm.

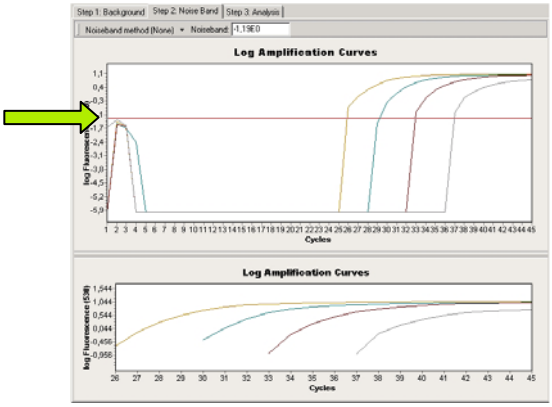
- Click on **Method (F.P)** in order to display the menu.



- Select **Fit points**. Three new tabs appear:
  Step 1 : Background
  Step 2 : Noise Band
  Step 3 : Analysis

- Click on **step 2 : Noise Band** to adjust the threshold line.

- The threshold line adjustment can be performed:
  - Automatically by clicking on **Noise Band** method and by selecting **Automatic**.
  - Manually by entering a threshold value in the suitable field or by positioning the threshold line on the value chosen.



- The threshold line adjustment must meet the following conditions:
  - The threshold line must be adjusted above the noise band.
  - The threshold line must cross all fluorescence curves (displayed in logarithmic mode) of samples in their linear part. If the threshold line cannot be positioned that way, repeat the analysis as many times as necessary.

- Click on **method (F.P)** and select **Automated (F'' max)** to go back to the automatic analysis mode through the second derivative method.

Only for quantification:

Standard curve: Quantification Measure

- The quantification measure must be performed with the **AUTOMATED F'' MAX** method (second derivative method).

- If the standard curve is created with all 4 quantification standards (QS1, QS2, QS3, QS4):

- A calculated concentration is displayed for each capillary from the column **CONC (COPIES /ML)**. Read the calculated concentration for each sample previously identified as positive.

- If the standard curve is imported from the QS3

- Click on the **STANDARD CURVE** tab, then on **USE EXTERNAL**.

- Choose a standard curve previously saved.
  Example: "HSV1 R-gene or HSV2 R-gene or VZV R-gene – Lot number-date".

- Read the calculated concentration for each sample previously identified as positive in the column **CONC (COPIES /ML)**.

CREATION OF THE EXTERNAL STANDARD CURVE

ONLY FOR QUANTIFICATION

- External standard curve must be created:
  - When the **R-gene®** kit is used for the first time.
  - With every new batch number of the kit.
  - When the standard curve has been created more than 3 months ago.
- The external standard curve is created with the 4 quantification standards (**QS1, QS2, QS3, QS4**).
- This curve can be stored and imported in subsequent runs when using products of the same batch number. However, the quantification standard **QS3** ALWAYS has to be created with every new run.
- The value of the **EFFICIENCY** and the **CP** of **QS3** must be between:

	69-002	69-003	69-004 71-015 71-016 71-017	69-100	69-010	69-013
Efficiency value	1.900 2.050	1.900 2.050	1.700 2.100	1.900 2.050	1.900 2.050	1.800 2.100
CP of QS3	28 - 32	30 - 35	31 - 35	**CMV:** 30 - 35 **HHV6:** 30 - 34	29 - 33	28 - 32

- If the value of the **EFFICIENCY** or the **CP** value of **QS3** does not correspond to these parameters, the curve cannot be validated as an external standard curve. A new curve has to be created with all four quantification standards (**QS1, QS2, QS3, QS4**).
- Store the external standard curve by selecting **STANDARD CURVE (IN RUN)** then **SAVE AS EXTERNAL**.
- Type the name of standard curve (*for example: "HSV1 R-gene or HSV2 R-gene or VZV R-gene – Lot number-date"*) and store it in the file **STD CURVE**.
- Repeat for each parameter to be analysed.

ANALYSIS OF CONTROLS (IC2sample, IC2W0)

- Click on the ABSOLUTE QUANTIFICATION tab.

- Check if 560 nm is selected in CHANNEL.

  ⚠ *71-101 (DICO Extra r-gene®, Argene) has to be read at 530 nm.*

- Check that the validated colour compensation file was activated.

- Read the calculated CP at 560 nm in the CP column for each sample inhibition control (**IC2Sample**) and compare its value to the CP value of the reference extraction+ inhibition control (**IC2W0**).

NOTE : **Do not read the CP of the quantification standards (QS), sensitivity control (SC), positive control (PC) and negative amplification control (R0) et 560 nm.**

The interpretation results are only based on the comparison between the CP obtained for each sample inhibition control (IC2sample) and the CP obtained with reference extraction + inhibition control (IC2W0).

Interpretation of Results

- Detailed interpretation is described in each corresponding datasheet.

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