ABI PRISM 7500
DNA R-gene® kits
Programming

Software Version 1.4

Please refer to StepOne guideline if using ABI PRISM 7500® version 2.0.1.

- **The use of these guideline is dedicated for Argene kits that mentioned the instrument ABI PRISM 7500 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		FAM and/or VIC end of the annealing

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 ABI PRISM 7500, Version 1.4 Software.

3. Check if NONE is selected in the field PASSIVE REFERENCE (see page 3) because amplification premixes do not contain PASSIVE REFERENCE.

ABI PRISM Programming
(i.e.: 7500 Software 1.4)

STARTING THE APPLICATION

- Switch on the computer, its screen and the ABI PRISM real-time PCR platform.
- Enter user name and password if necessary.
- Click on the icon **7500 SYSTEM SOFTWARE** (Fig.1).

Fig.1

- A **QUICK STARTUP DOCUMENT** window appears (Fig.2).
- Select **CREATE NEW DOCUMENT...**

  A **NEW DOCUMENT WIZARD** window opens (Fig.3).

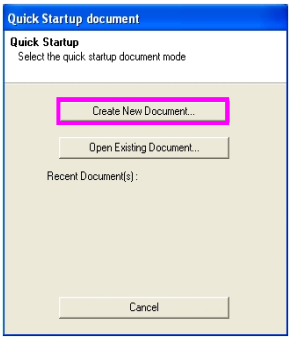


Fig.2

DEFINE DOCUMENT

- Make sure that:
  - In Assay field, **STANDARD CURVE (ABSOLUTE QUANTITATION)** is selected.
  - In Container field, **96-WELL CLEAR** is selected.
  - In Template field, **BLANK DOCUMENT** is selected.
  - In Run Mode, **STANDARD 7500** is selected.
  - Click on **NEXT**.

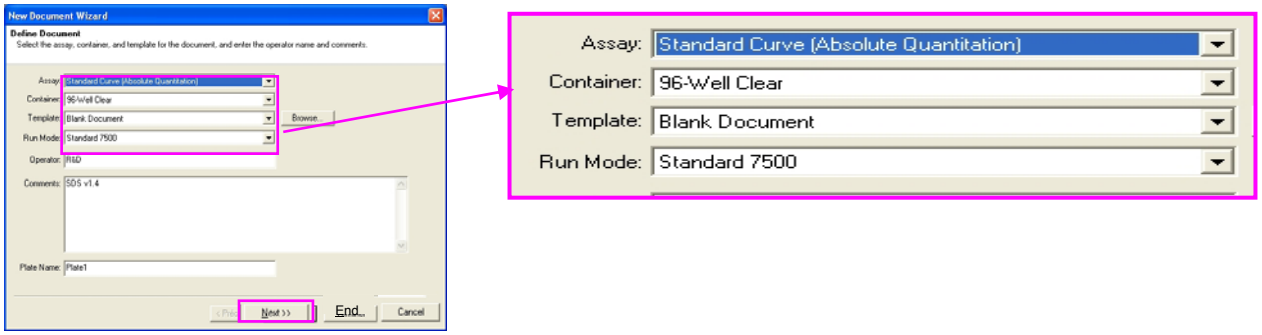


Fig.3

CREATE DETECTORS (If you run the program for the first time).

- Click on NEW DETECTOR... and enter the following data (Fig.4) :
  - In the NAME field enter FAM RGENE (Fig.5).
  - Leave the field DESCRIPTION blank.
  - In the field REPORTER DYE enter FAM.
  - In the field QUENCHER DYE enter (NONE).
  - Click on the COLOR field and choose green (Fig.6).
  - Click on OK to validate the creation of the detector FAM RGENE.

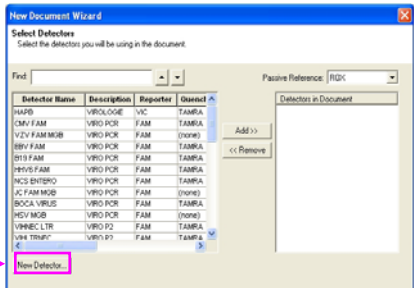


Fig.4

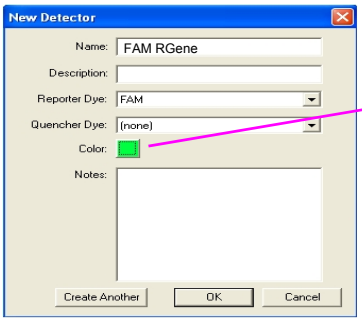


Fig.5

Fig.6

- In the same way, click again on NEW DETECTOR... and enter the following data (Fig.7):
  - In the field NAME enter VIC RGENE.
  - Leave the DESCRIPTION field blank.
  - In the REPORTER DYE field enter VIC.
  - In the QUENCHER DYE field enter (NONE).
  - Click on the COLOR field and choose YELLOW.
  - Click on OK to validate the creation of the detector VIC RGENE.

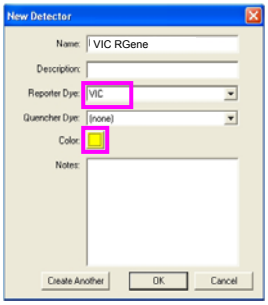


Fig.7

- The two detectors created appear in the list of detectors (Fig.8).
- Select the detectors FAM R-GENE and VIC R-GENE and click on ADD >>.
- The two detectors selected appear now in the field DETECTORS IN DOCUMENT (Fig.8).
- Select (NONE) in PASSIVE REFERENCE (amplification premix does not contain passive reference).
- Click on NEXT.

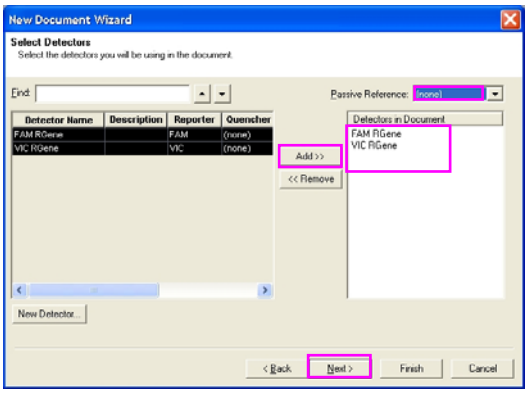


Fig.8

SET UP SAMPLE PLATE

- Tick the checkboxes of the two detectors previously selected.

- Click on **FINISH** (Fig. 9).

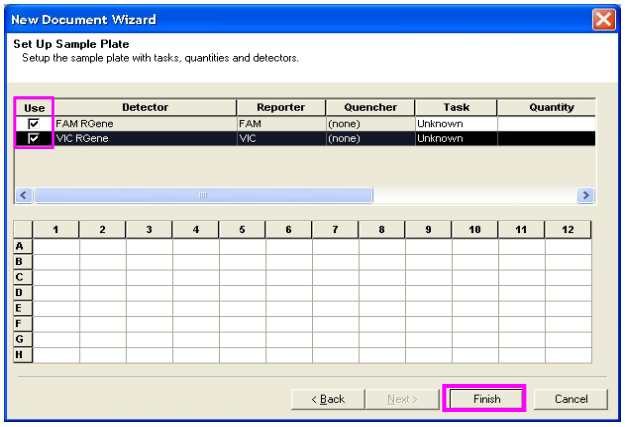


Fig.9

ENTRY OF THE PROGRAM DATA

- Click on the **INSTRUMENT** tab (Fig.10).

- Double-click on **STAGE 1** part and click on **DELETE** (Fig11).

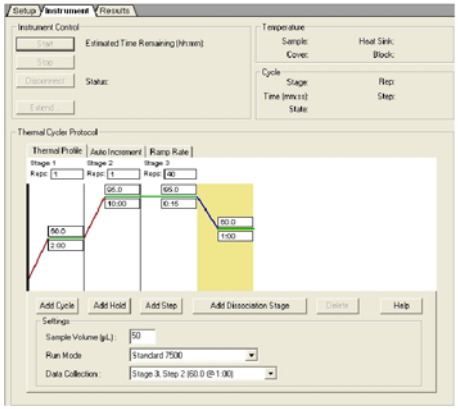


Fig.10

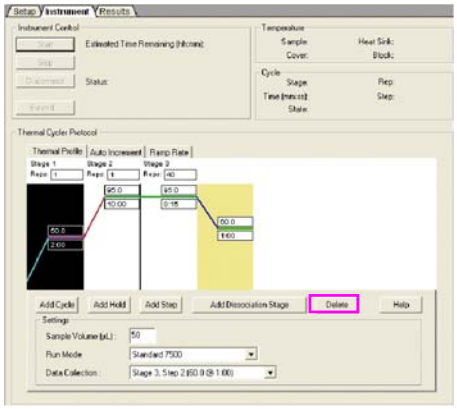


Fig.11

Hot Start Taq Activation:

Click on the part **STAGE 1** and enter the value **95.0** in the top part of the window.
Then, enter the value **15:00** in the bottom part of the window (Fig.12).

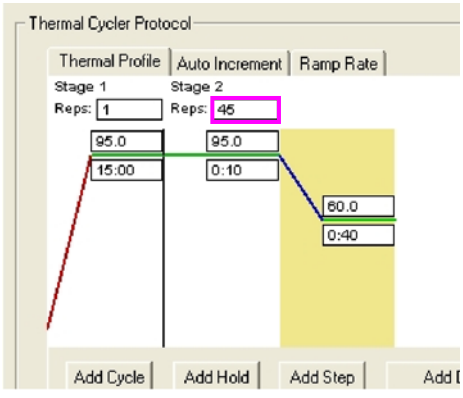


Fig.12

Denaturation step:

Click on the left part of the **STAGE 2**.
Enter the value **95.0** in the top part of the window.
Enter the value **0:10** in the bottom part of the window.

Annealing/Elongation step:

In the right part of the **STAGE 2** :
Enter the value **60.0** in the top part of the window.
Enter the value **0:40** in the bottom part of the window.

- **STAGE 2** is repeated 45 times, so type **45** in the blank field **REPS**.

- Click on the **Ramp Rate** tab (Fig.13).

- Check that the parameter **100%** appears in the second step of the cycling stage (step at 60°C).

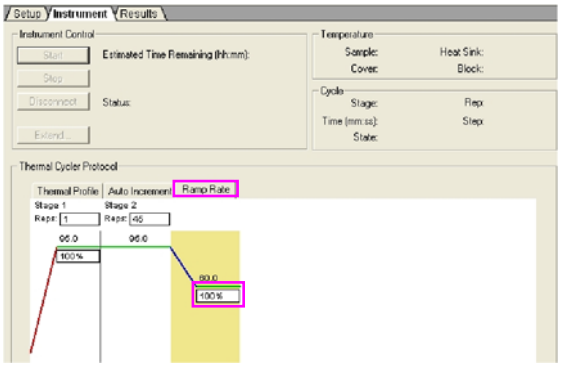


Fig.13

- In **Settings** field enter **25** in **Sample Volume (µL)** (Fig.14).
- In **Run Mode** select **Standard 7500**.
- In **Data collection** select **Stage2, Step2 (60.0@0:40)**.
  (Data collection step selected appears in colour.)

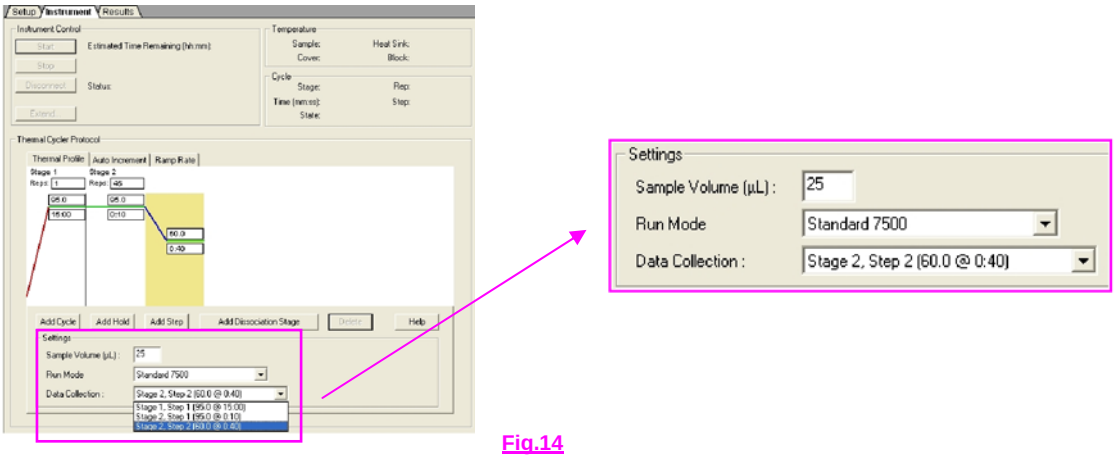


Fig.14

To save the program:

- Click on **File** in the Menu bar and **Save as** (Fig. 15).
- Save your file in **Templates** as **60°Rgene** and check that **SDS Templates (*.sdt)** is selected in **Type field**.

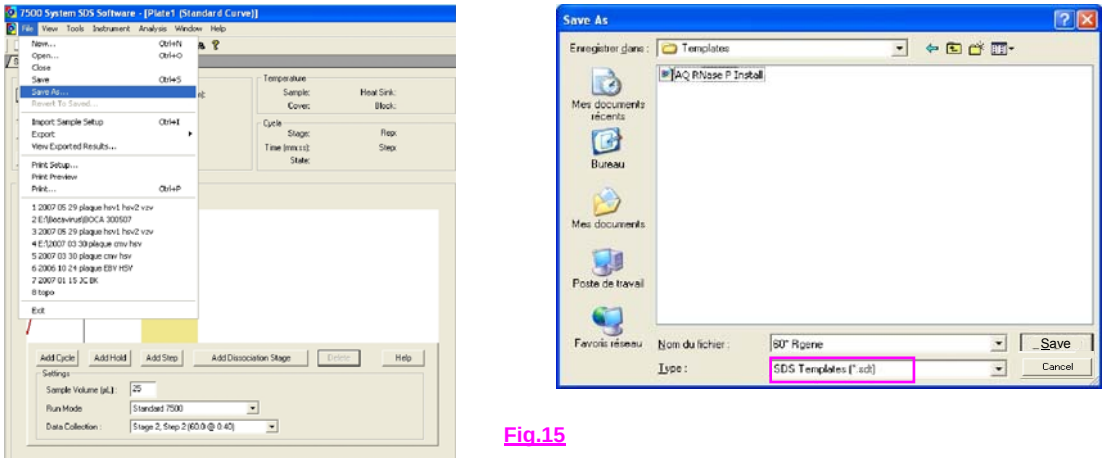
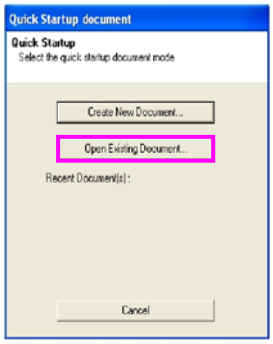


Fig.15

Running the Program

RUNNING THE PRE-STORED PROGRAM

- In the home page of the software when the QUICK STARTUP window appears:
  - Select OPEN EXISTING DOCUMENT and choose 60° RGENE in Templates file (Fig.16).



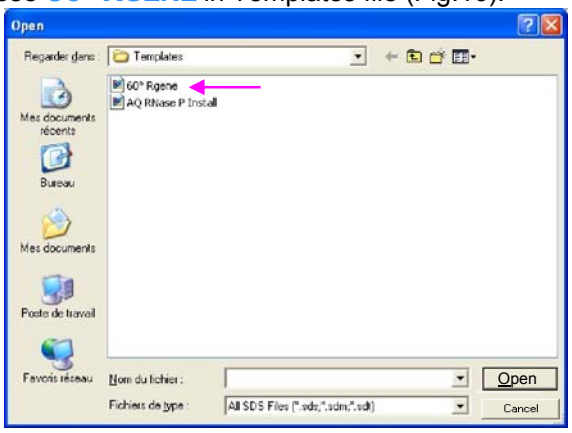


Fig.16

DEFINE THE SAMPLES

- Click on the SET UP tab (Fig.17).
- For each well to define:
  - Double click on the well :
- A new window WELL INSPECTOR appears:
- According to the table below :
  - Select the checkbox(es) of the appropriate(s) detector(s): FAM RGENE and/or VIC RGENE.
  - Enter the name of the sample in SAMPLE NAME field.
  - Specify the samples in the TASK column.
  - Specify the concentration of the quantification standard in the QUANTITY column (cf table).
  - Click on CLOSE.

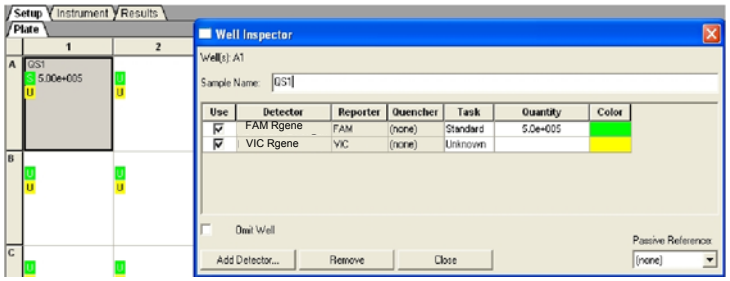


Fig.17

	Quantitative kits		Qualitative kits	
Channels	FAM	VIC	FAM	VIC
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**)	NTC (NO TEMPLATE CONTROL)	UNKNOWN	NTC (NO TEMPLATE CONTROL)	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:

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 / QIAcube	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
		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 50 µL)	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 / QIAcube	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
		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 150 µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP	Plasma	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	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®	QIAcube	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						
	MagNA Pure Compact MagNA Pure LC System		CSF						
	NucliSENS® easyMAG®		CSF						
	Versant kPCR Molecular System SP		CSF	400 µL (extract 250 µL)	65 µL (eluate 50 µL)				
69-010 ADENOVIRUS R-gene®	QIAcube	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
		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 150 µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP		Plasma / CSF	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	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 / QIAcube	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
		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 50 µL)	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.

- Click on **INSTRUMENT TAB**, click **CONNECT** (if necessary), then insert the microplate in the instrument.
- Launch the run by clicking on **START** (Fig. 18).

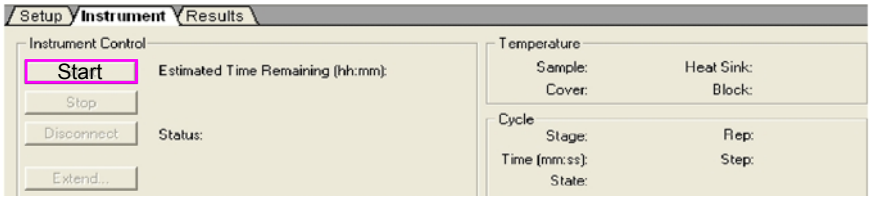


Fig.18

Data Analysis

- At the end of the experiment, the **SDS** software automatically opens.
- Click on **OK**.
- Click on **ANALYSIS** tab (Fig.19) then on **ANALYZE** (or click on this button  .)

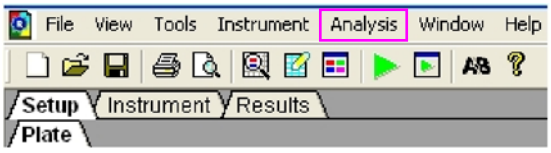


Fig.19

- Click on **RESULTS** tab then **AMPLIFICATION PLOT**.

LINEAR MODE: QUANTIFICATION OF THE SAMPLES

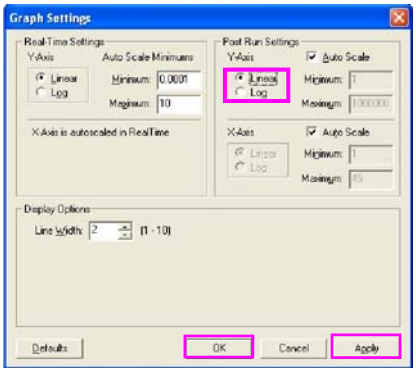


Fig.20

- In TOOLS in the menu bar, select GRAPH SETTINGS...
- In POST RUN SETTINGS field (Fig.20) :
  - In the Y-AXIS tab, select LINEAR radio button.
  - Click on APPLY then OK.
- Mark all samples in the table that you want to analyze.
- In DATA field select DELTA RN VS CYCLE (Fig.21).
- In DETECTOR field select FAM RGENE.

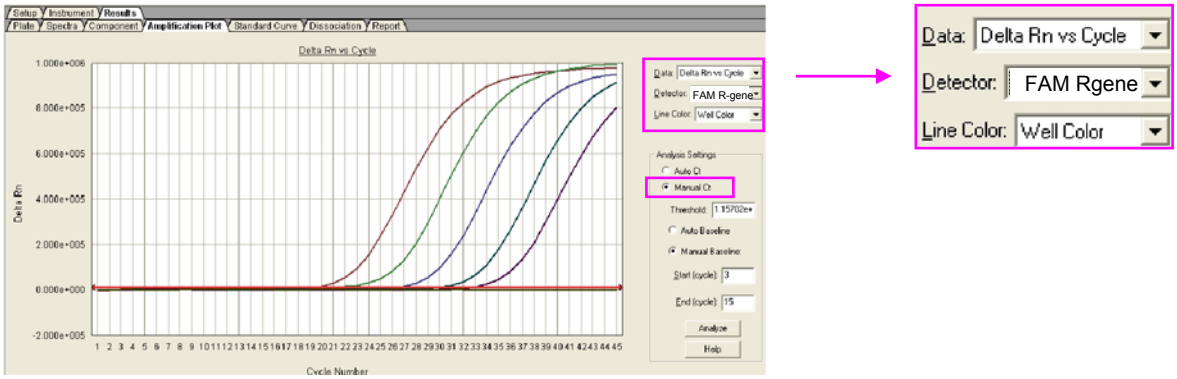


Fig.21

MANUEL BASELINE ADJUSTMENT : IDENTIFICATION OF THE SAMPLES

- Select MANUAL CT (Fig.22) and adjust the noise band (green horizontal line) to a position where it crosses the fluorescence curves of all the samples in their exponential part (Fig.23).
- Click on ANALYZE.

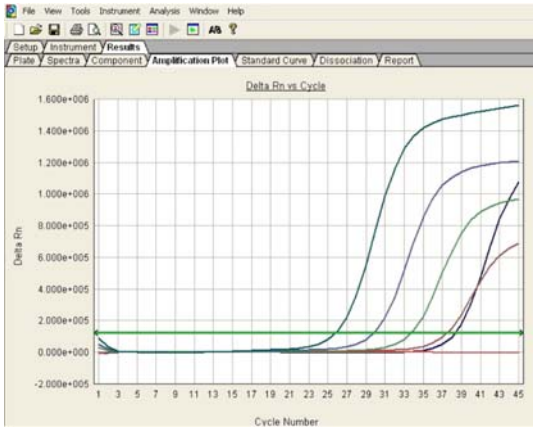


Fig.22

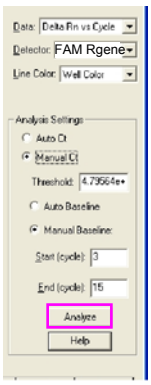


Fig.23

ONLY FOR QUANTIFICATION

- Click on STANDARD CURVE tab to obtain the slope of the standard curve (Fig.24).

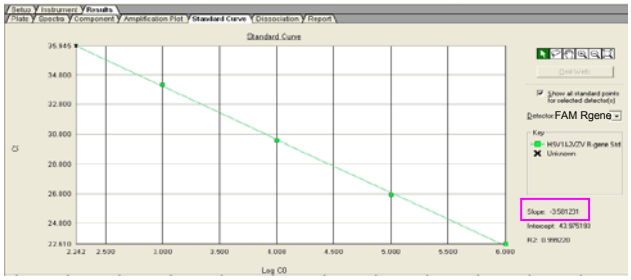


Fig.24

- The quantification results are edited in the REPORT tab.

ANALYSIS OF CONTROLS (IC2sample, IC2W0)

- Return in the AMPLIFICATION PLOT tab.

- Click on the DETECTOR field then select the detector VIC R-GENE (Fig.25).

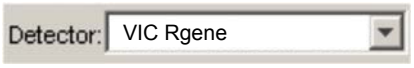


Fig.25

- Perform the manual baseline adjustment as described in the previous section.

- Read the calculated CT in the REPORT tab at VIC R-GENE line for each sample inhibition control (**IC2sample**) and compare its value to the CT value of the reference extraction + inhibition control (**IC2W0**).

NOTE : **Do not read the CT of the quantification standards (QS), sensitivity control (SC), positive control (PC) or negative amplification control (R0) with the VIC R-GENE detector (VIC channel).**

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

Interpretation of Results

- Detailed interpretation is described in each corresponding datasheet.

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BIOMÉRIEUX

bioMérieux S.A.
Chemin de l'Orme
69280 Marcy-l'Étoile - France

RCS LYON 673 620 399
Tel.: 33 (0)4 78 87 20 00
Fax: 33 (0)4 78 87 20 90
www.biomerieux.com