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RT-06
quanty Measles on LightCycler 480

INTERNAL CONTROL

To assure the correct detection of the target, Clonir srl declare to add in the extraction phase an internal control to monitor all the reaction phases.
In the amplification reaction of each sample, the Ct values for the internal control are used for validating the analysis session beginning from extraction process until detection stage.
A good extraction performances presents internal control threshold cycle < 35
Be sure that emitted fluorescence from internal control amplification has not a Ct > 35 or undetermined. If a sample presents an undetermined **Measles** RNA and internal control Ct >35 means that some problems happened in the extraction stage or in the amplification stage; therefore the sample could be a false negative. **Repeat the sample**.
If there is not possible to add the Internal Control at the beginning of the test, it is so possible to add 1µl/test of Internal Control in the reconstituted Master Mix dedicated to the samples.
In this way it will be possible to monitor the amplification phase and the absence of PCR inibitor due to a wrong extraction, but it is not possible to monitor the success of the sample extraction.

SOFTWARE SETTING:

LightCycler 480

Turn the instrument and the computer on and start the control software. In the principal screen, select "**Open Tools**" (wrench image), click on "**Detection formats**" and set a NEW filter combination selection: flag the wavelenght dedicated 553-580 (VIC) and 618-660 (CY5) and give the Name **"VIC CY5"**

Target	Reporter
Measles probe:	VIC
Internal Control (IC) probe:	CY5

On "**Experiment Creation** push the "**New experiment**" button. The window "experiment" appears. On the "Run protocol" sheet set:
1- Detection format: VIC-CY5
2- Reaction volume: 30 µl
3- Thermal Protocol

Cycles	Retrotrascription	Denaturation	Annealing	Extension
1	50° C 40 min			
1		95°C 2 min		
45		95° C 15 sec	58° C 45 sec	72° C 15 sec

Set the acquisition mode on "SINGLE" in annealing phase.

Push the "**Subset editor**" button and in this window. Create a New Subset clicking in (+) button and rename it as Measles. Select an area of the plate where controls and samples will be placed and click **Apply**.
Push the "**Sample editor**" button. Select the correct workflow (Step1: **Abs quantification**), choose the samples Subset created in the step before and insert the name for each well. Choose the correct type for each well: Standard, Negative CTR or Unknown sample.
Set the correct concentration for all the standards

By correctly setting the standards concentration as a function of the extraction system you can get the quantization of the sample directly in copies/ml:

		Copie/react
	RTS 1	1.500.000
	RTS 2	150.000
	RTS 3	15.000
	RTS 4	1.500

Sample concentration expressed in copies/ml will be obtained using the formula:

$$copie/ml = \frac{1000}{Ve} \times \frac{Ev}{Ea} \times C_{reaz}$$

where:
- **Ve:** extracted sample Volume expressed in μl
- **Ev:** eluted sample Volume during extraction stage expressed in μl
- **Ea:** extracted sample volume used for amplification expressed in μl

C_{reaz}: copies provided by the instrument

Push again the "**experiment button**", insert the plate in the instrument and push "**start run**".

When the run is completed select analysis and choose the correct kind of analysis you want: "**Abs Quant/Fit Points**". Choose the samples subset you want to analyze. Select the "**NoiseBand**" sheet, under the plot you can choose "**NoiseBand (Fluoresc.)**"; and move the line of the NoisBand on the plot with the mouse of your PC. Repeat this action for each fluorophore using the "**Filter comb"** button.
Clicking the sheet "**Analysis**" you can set the threshold choosing the option "Threshold(manual).

After setting parameters push the "**Calculate**" button. Repeat this action for each fluorophore.

See paragraph **"INTERPRETATION OF RESULTS"**

17.01.2020