ViralQSearch™ Plus

RCL qPCR Detection Kit (EP)

Version:2.0

Latest update: 2024.02.01

Instruction Manual

Cat. No. RCLP-100

Research Use Only. Not for Use in Diagnostic Procedures.

Contents

1. Product Information

Introduction

The Lentiviral vector system is a useful tool for transferring therapeutic genes into cells for the treatment of human diseases. It is ideal for delivering the target gene to host cells and stably inserting it into the chromosome. However, it poses a potential risk to human health due to the generation of Replication Competent Lentivirus (RCL), which can infect non-target cells.

Considering the risk of Replication Competent Lentivirus (RCL), all cell gene therapies based on the Lentiviral vector system should undergo testing for the presence of RCL before patient treatment. Current FDA guidelines regarding RCL recommend the use of cell-based assays. However, Cell-based assays may require up to six weeks to obtain results. Therefore, qPCR-based analysis serves as an alternative for rapidly assessing the presence of RCL.

Kit Specificity

The ViralQsearch™ Plus RCL(Replication Competent Lentivirus) qPCR Detection Kit can detect only RCL simply, quickly, and reproducibly. This Kit uses a PCR (Polymerase Chain Reaction)-based method to specifically detect RCL without the influence of host DNA.

Kit Sensitivity

The ViralQsearch™ Plus RCL (Replication Competent Lentivirus) qPCR Detection Kit possesses a high sensitivity, capable of detecting the target gene at levels ranging from 1 to 10 copies/reaction. The sensitivity to cell culture samples may vary depending on the quality of the sample preparation process. Importantly, it maintains its detection capability even in the presence of high concentrations of genomic DNA within the sample, ensuring reliable results.

Prevention of carryover contamination

When performing PCR, care must be taken to ensure that the amplicon created in the previous PCR does not enter the next PCR reaction. The 2X qPCR master mix included in the product contains dUTP instead of dTTP. When dUTP replaces dTTP in PCR amplification, UNG treatment (Uracil-N-glycosylase, HiSense™ UDG heat labile, Cat.No.SCU-100, not provided in this kit) can prevent the subsequent reamplification of dU-containing PCR products. UNG acts on single- and double-stranded dU-containing DNA by hydrolysis of uracil-glycosidic bonds at dU-containing DNA sites. With this strategy, carryover contamination is eliminated, while the template DNA (DNA containing T) remains intact.

Precaution for cross contamination

To prevent cross contamination, use separate locations and pipettes for handling positive controls during the PCR process. Additionally, wear gloves during experiments and use filter tips to prevent aerosols.

2. Contents and Storage

Materials Provided

Label	Cap	Content
2X qPCR Master Mix	Blue	1.25 ml
Primer and Probe Mix	Amber	200 µl
Internal Amplification Control DNA	Orange	200 µl
Internal Amplification Control DNA for sample prep	Violet	2 ml
Positive Control DNA	Yellow	50 µl
50X Low ROX*	Amber	60 µl
DNase Free Water	White	1.2 ml

* If High ROX is required depending on equipment, it will be provided separately.

ROX concentration for Instruments: See [Appendix A]

Storage

Upon receipt, store at -20°C.

Note

1) Repeat thawing reduces quality of product.

2) If frequent freeze and thaw is needed, aliquot the products and use in order.

3) Check the label on the product for details.

3. Test protocol

Prepare the Template (Sample)

Samples should be derived from cultures which are at 90~100% confluence. Penicillin and streptomycin in the culture media do not inhibit PCR or affect test sensitivity. To avoid false positive results, we recommend the use of the PCR grade water delivered with the kit, aerosol-preventive filter tips and gloves. DNA samples are prepared using a commercially available kit and proceed according to the method provided by the kit.

The preparation of sample for validity DNA extraction

1) Follow the manual for the DNA extraction kit you are using, and add 20 µl of Internal Amplification Control DNA for sample prep (Violet cap) at the step of adding Proteinase K.

2) In the elution step, 50 µl of the elution volume is obtained, and the genomic DNA is used as a PCR template.

Prepare for qPCR

1. Prepare the set of reactions listed in the following table.

(Caution!! Don't vigorous vortexing.)

Reaction Components		Sample Reation	Positive Control Reaction	No Template Control Reaction
Label	Cap			
2X qPCR Master Mix	Blue	12.5 µl	12.5 µl	12.5 µl
Primer and Probe Mix	Amber	2 µl	2 µl	2 µl
Internal Amplification Control DNA	Orange	- *	2 µl	2 µl
Template DNA	-	5 µl	-	-
Positive Control DNA	Yellow	-	1 µl	-
50X Low ROX **	Amber	0 µl(No ROX), 0.5µl (Low ROX) **		
DNase Free Water	White	Up to 25 µl		
Final volume		25 µl	25 µl	25 µl

* In the sample reaction, internal amplification control DNA is not added separately because the sample already include internal amplification control DNA through the sample preparation of Genomic DNA extraction procedure.

** ROX concentration for Instruments: See [Appendix A]

2. Set up the qPCR instrument to run the PCR cycling (amplification) program specified below.

Steps & Cycle			Temp(℃)	Time
Pre Heat			95	5 min
PCR	45 Cycles	Denature	95	10 sec
		Anneal	60	20 sec
		Extend*	72	20 sec**
		*Acquisition RCL DNA – FAM (470~510 nm), green channel Internal Control DNA – HEX or VIC (535~559 nm), yellow channel Positive Control - FAM (470~510nm) and Cy5 (650~670 nm)		

** For ABI 7500 and 7500 Fast Instrument, set Extension time to 30 sec.

4. Results / Trouble Shooting

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in HEX channel for internal control. The presence of RCL DNA in the sample is detected through an increasing fluorescence signal in the FAM channel. Cross contamination of positive control DNA during the experiment is identified using the Cy5 channel.

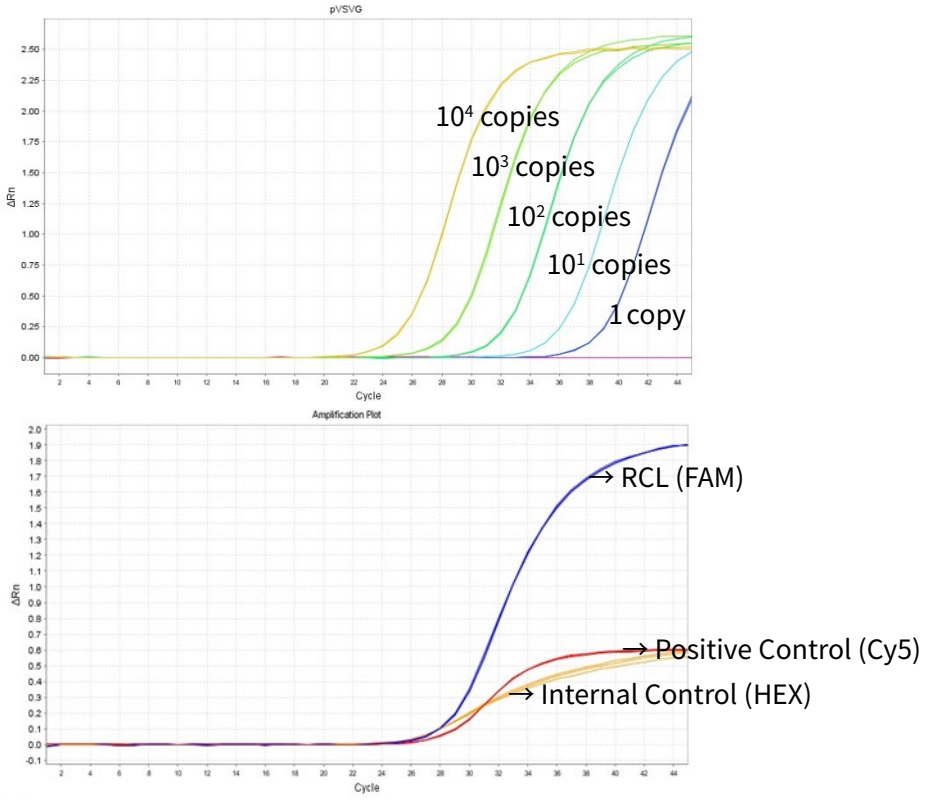
False-negative results, resulting from the inhibition of the PCR reaction by the sample matrix, can be identified individually for each sample, as these reactions do not exhibit any fluorescence signal. Refer to the table below for the interpretation of the test results.

FAM channel (RCL DNA)	Cy5 channel (Positive control DNA)	HEX or VIC channel (Internal control DNA)	Interpretation
Positive (Ct value < 40)	Positive	Positive	Positive control DNA contamination
Positive (Ct value < 40)	Positive	Negative*	Positive control DNA contamination
Positive (Ct value < 40)	Negative	Positive	RCL Positive
Positive (Ct value < 40)	Negative	Negative*	RCL Positive
Negative	Negative	Positive	RCL Negative
Negative	Negative	Negative	PCR inhibition

* HEX is not detected when there is a significant amount of RCL DNA.

Amplification Result

Tested with Quantitative VSVG Plasmid DNA (Cat. No. QVPD, CellSafe) in the presence of 1µg of Human Genomic DNA.



[Appendix A] ROX concentration for Instruments

Instruments		Reference dye
Brand	**Model**	
BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384	No ROX
MJ Research	Opticon, Option2, Chromo4, MiniOpticon	No ROX
Qiagen	Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000	No ROX
Eppendorf	Mastercycler realplex	No ROX
Illumina	Eco RealTime PCR System	No ROX
Roche	LIghtCycler 480, LightCycler 2.0	No ROX
ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX
ABI	7500, 7500 Fast, QuantStudio (3, 5, 7)	Low ROX
Stratagene	MX3000, MX3005P, MX4000	Low ROX