

Viral Genome Release and Native Viral Particle Conversion Measured Through Temperature Ramping in Real Time PCR Machine

Antonio Real-Hohn

Abstract

This protocol was developed to use common wet lab device (Real Time PCR machine) to detect viral genome release and native viral particle conversion. We used temperature ramping for inducing genome uncoating and fluorescent probes for RNA/DNA and/or proteins, to detect genome and/or capsid changes.

Citation: Antonio Real-Hohn Viral Genome Release and Native Viral Particle Conversion Measured Through Temperature Ramping in Real Time PCR Machine. **protocols.io**

dx.doi.org/10.17504/protocols.io.p2xdqfn

Published: 10 May 2018

Guidelines

Prepare a master mix (3.5X) with virus and probes in thermo stable buffer

Final volume 70 μ L

Add replicates to 96-well PCR plate (Replicates 3 X 20 μ L)

Seal the plate

Place the plate in Real Time PCR Equipment

Open the software and run a melting curve assay.

Materials

500g Potassium Phosphate (Dibasic) [RC-081](#) by [G-Biosciences](#)

500g Potassium Phosphate (Monobasic) [RC-083](#) by [G-Biosciences](#)

SYTO 82 Orange Fluorescent Nucleic Acid Stain [S11363](#) by [Thermo Fisher Scientific](#)

SYPRO Orange Protein Gel Stain [S6650](#) by [Thermo Fisher Scientific](#)

Protocol

Mix Itens in 200 µL tubes and then transfere to a 96-well Real Time PCR Plate

Step 1.

Purified Virus (Rhinovirus, Poliovirus, Coxsackie-virus, etc.)

AMOUNT

5 µg Additional info:

TEMPERATURE

4 °C Additional info:

Mix Itens in 200 µL tubes and then transfere to a 96-well Real Time PCR Plate

Step 2.

Fluorescent Probe for DNA/RNA (SYTO-82) and/or Fluorescent Probe for Protein (SYPRO Orange)

CONCENTRATION

0.000005 Molarity (M) Additional info:

TEMPERATURE

4 °C Additional info:

Mix Itens in 200 µL tubes and then transfere to a 96-well Real Time PCR Plate

Step 3.

Viral Thermo Stable Buffer (Phosphate, Cacodylate, etc.)

Potassium Phosphate Buffer

CONCENTRATION

0.1 Molarity (M) Additional info:

TEMPERATURE

4 °C Additional info:

Run a melting curve program in the equipment software. Starting with 25 °C for 5 minutes and ramping 0.5 °C degrees each 5 seconds.

Step 4.

Temperature Ramping

TEMPERATURE

25 °C Additional info: Start

TEMPERATURE

95 °C Additional info: End

EQUIPMENT

Equipment brand:

CFX Connect Real-Time PCR Detection System BioRad

SKU:

MFPL

Specifications:

Step 5.

Warnings

All the material must be decontaminated before trashing.