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

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Protocol status: Working
We use this protocol and it's working

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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.

Guidelines

Prepare a master mix (3.5X) with virus and probes in thermo stable buffer
Final volume 70 µL
Add replicates to 96-well PCR plate (Replicates 3 X 20 µL)
Seal the plate
Place the plate in Real Time PCR Equipment
Open the software and run a melting curve assay.

Materials

MATERIALS

- 500g Potassium Phosphate (Dibasic) **G-Biosciences Catalog #**RC-081
- 500g Potassium Phosphate (Monobasic) **G-Biosciences Catalog #**RC-083
- SYTO 82 Orange Fluorescent Nucleic Acid Stain **Thermo Fisher Scientific Catalog #**S11363
- SYPRO Orange Protein Gel Stain **Thermo Fisher Scientific Catalog #**S6650

Safety warnings

All the material must be decontaminated before trashing.

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

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

5 µg

4 °C

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

[M] 0.000005 Molarity (M)

4 °C

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

[M] 0.1 Molarity (M)

4 °C

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.

4 Temperature Ramping

Equipment	
new equipment	NAME
CFX Connect Real-Time PCR Detection System BioRad	BRAND
MFPL	SKU

25 °C Start

95 °C End

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