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 We use this protocol and it's working

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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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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

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⊗ 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 R...

- 1 Purified Virus (Rhinovirus, Poliovirus, Cocksackie-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. St..

- 4 Temperature Ramping

Equipment

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

🌡️ 25 °C Start

🌡️ 95 °C End

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