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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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**Keywords:** temperature ramping in real time pcr machine, native viral particle conversion, viral genome release, real time pcr machine, fluorescent probes for rna, inducing genome uncoating, pcr, temperature ramping, common wet lab device, genome uncoating, time pcr, fluorescent probe, temperature

## 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  $\mu$ L

Add replicates to 96-well PCR plate (Replicates 3 X 20  $\mu$ L)

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**

## Troubleshooting

## Safety warnings

! All the material must be decontaminated before trashing.



## Mix Itens in 200 $\mu$ L tubes and then transfere to a 96-well Real Time PCR Plate

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

5  $\mu$ g

4  $^{\circ}$ C
- 2 Fluorescent Probe for DNA/RNA (SYTO-82) and/or Fluorescent Probe for Protein (SYPRO Orange)  

[M] 0.000005 Molarity (M)

4  $^{\circ}$ C
- 3 Viral Thermo Stable Buffer (Phosphate, Cacodylate, etc.)  
Potassium Phosphate Buffer  

[M] 0.1 Molarity (M)

4  $^{\circ}$ C

Run a melting curve program in the equipment software. Starting with 25  $^{\circ}$ C for 5 minutes and ramping 0.5  $^{\circ}$ C degrees each 5 seconds.

- 4 Temperature Ramping

### Equipment

new equipment

NAME

CFX Connect Real-Time PCR Detection System BioRad

BRAND

MFPL

SKU

25  $^{\circ}$ C Start

95  $^{\circ}$ C End

- 5