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A validated protocol to UV-inactivate SARS-CoV-2 and herpesvirus-infected cells V.2

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✓ Peer-reviewed method

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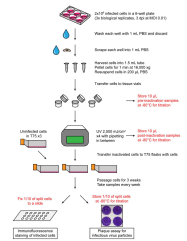
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Protocol status: Working

We use this protocol and it's working

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Abstract

Downstream analysis of virus-infected cell samples, such as reverse transcription polymerase chain reaction (RT PCR) or mass spectrometry, often needs to be performed at lower biosafety levels than their actual cultivation, and thus the samples require inactivation before they can be transferred. Common inactivation methods involve chemical crosslinking with formaldehyde or denaturing samples with strong detergents, such as sodium dodecyl sulfate. However, these protocols destroy the protein quaternary structure and prevent the analysis of protein complexes, albeit through different chemical mechanisms. This often leads to studies being performed in over-expression or surrogate model systems. To address this problem, we generated a protocol that achieves the inactivation of infected cells through ultraviolet (UV) irradiation. UV irradiation damages viral genomes and crosslinks nucleic acids to proteins but leaves the overall structure of protein complexes mostly intact. Protein analysis can then be performed from intact cells without biosafety containment. While UV treatment protocols have been established to inactivate diluted viral solutions, a protocol was missing to inactivate crude infected cell lysates, which heavily absorb light. In this work, we develop and validate a UV inactivation protocol for SARS-CoV-2, HSV-1, and HCMV-infected cells. A fluence of 10,000 mJ/cm² with intermittent mixing was sufficient to completely inactivate infected cells, as demonstrated by the absence of viral replication even after three sequential passages of cells inoculated with the treated material. The herein described protocol should serve as a reference for inactivating cells with these or similar viruses and allow for the analysis of protein quaternary structure from *bona fide* infected cells.

The **last step** in this version contains a supplemental video with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

Guidelines

This protocol was validated for 2×10⁶ Vero E6 cells infected with SARS-CoV-2 at MOI 0.01, 5×10⁵ Vero B4 cells infected with HSV-1 at MOI 3, and 2×10⁵ HFF-1 cells infected with HCMV at MOI 3 in an individual 6-well.

Materials

phosphate-buffered saline (PBS)
Incidin Plus

infected cells
cell scraper
microcentrifuge tube
CryoELITE Tissue Vial (Wheaton, #W985100)

P1000 micropipette
microcentrifuge
UVP Crosslinker (CL-3000, Analytik Jena)



Safety warnings

- ⚠ Handle infectious materials within the appropriate containment facilities. Use UVP Crosslinkers in accordance with the manufacturer's guidelines.

Before start

Begin with infected cells in a 6-well plate.



UV inactivate infected cells

- 1 Wash each well with 1 mL PBS 1m
- 2 Scrape each well (2×10^6 cells) into 1 mL PBS 1m
- 3 Transfer cells into a 1.5 mL tube 1m
- 4 Pellet cells at  16000 x g, 4°C, 00:01:00 1m
- 5 Resuspend cells in 200 µL PBS 1m
- 6 Transfer cells to a tissue vial 1m
- 7 254 nm UV irradiation of vials 24m
- 7.1 Irradiate vials with 2,500 mJ/cm² 5m
- 7.2 Mix the cell solution with a micropipette 1m
- 7.3 Repeat irradiation 3 additional times for a total of 10,000 mJ/cm² 18m
- 8 Screw the lids on the vials 1m
- 9 Disinfect the outside of the tissue vials by wiping with Incidin Plus 1m



Spotlight video

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<https://www.youtube.com/embed/-EGddr76w7U?si=3yCahkyNGpYFLY4T>