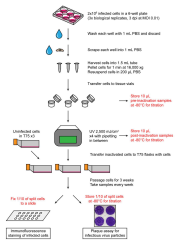
May 11, 2023 Version 1

A validated protocol to UV-inactivate SARS-CoV-2 and herpesvirus-infected cells V.1



PLOS One

Peer-reviewed method

DOI
dx.doi.org/10.17504/protocols.io.81wgb676qlpk/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.81wgb676qlpk/v1

External link: https://doi.org/10.1371/journal.pone.0274065

Protocol Citation: Timothy K. Soh, Susanne Pfefferle, Stephanie Wurr, Ronald von Possel, Lisa Oestereich, Toni Rieger, Maria Rosenthal, Jens B. Bosse 2023. A validated protocol to UV-inactivate SARS-CoV-2 and herpesvirus-infected cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgb676qlpk/v1

Manuscript citation:
Soh TK, Pfefferle S, Wurr S, Possel Rv, Oestereich L, Rieger T, Uetrecht C, Rosenthal M, Bosse JB (2023) A validated protocol to UV-inactivate SARS-CoV-2 and herpesvirus-infected cells. PLoS ONE 18(5): e0274065. doi: 10.1371/journal.pone.0274065

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

Created: January 19, 2022

Last Modified: May 11, 2023

Protocol Integer ID: 57133

Keywords: SARS-CoV-2, herpes, HSV-1, HCMV, UV crosslinking, UV inactivation, inactivation validation, uv inactivation protocol for sar, uv inactivation protocol, uv treatment protocol, uv irradiation, diluted viral solution, inactivation of infected cell, analysis of protein complex, validated protocol to uv, overall structure of protein complex, nucleic acids to protein, protein complex, common inactivation method, analysis of protein, chemical crosslinking with formaldehyde, herpesvirus, protein, inactivate sar, infected cell sample, such as reverse transcription polymerase chain reaction, protein analysis, viral genome, analysis of virus, uv, reverse transcription polymerase chain reaction, ultraviolet, absence of viral replication, intact cells without biosafety containment, chemical crosslinking, infected cell lysate, nucleic acid, infected cell, protein quaternary structure, irradiation, such as sodium dodecyl sulfate, reverse transcription, viral replication

Funders Acknowledgements:
Deutsche Forschungsgemeinschaft
Grant ID: EXC 2155 – project number 390874280
Deutsche Forschungsgemeinschaft
Grant ID: GRK 2771 project number 453548970
Wellcome Trust
Grant ID: Collaborative Award (209250/Z/17/Z)
Bundesministerium für Bildung und Forschung
Grant ID: grant 01KI2019

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/cm^2 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.

Guidelines

This protocol was validated for 2×10^6 Vero E6 cells infected with SARS-CoV-2 at MOI 0.01, 5×10^5 Vero B4 cells infected with HSV-1 at MOI 3, and 2×10^5 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)

Troubleshooting

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/cm^2 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/cm^2 18m

8 Screw the lids on the vials 1m

9 Disinfect the outside of the tissue vials by wiping with Incidin Plus 1m