



cebataie ▾

Collection: State-of-the-Art Analytical Methods of Viral Infections in Human Lung Organoids V.(cebataie)

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1 Works for me

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DISCLAIMER

Informed written consent was obtained from all volunteers and the study was approved by the Charité Ethics Committee (project 451, EA2/079/13).

ABSTRACT

Organ models have received widespread attention in the study of SARS-CoV-2, the pathogen causing the current COVID-19 pandemic. Human-based organ models can provide strong predictive value to investigate the tropism, virulence, and replication kinetics of viral pathogens.

Applicable to a large set of organoid models and viruses, we provide a step-by-step work instruction for the infection of human alveolar-like organoids with SARS-CoV-2 in this protocol collection. We also prepared a detailed description on state-of-the-art methodologies to assess the infection impact and the analysis of relevant host factors in organoids.

This protocol collection consists of five different sets of protocols. Set 1 describes the protein extraction from human alveolar-like organoids and the determination of protein expression of angiotensin-converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2) and FURIN as exemplary host factors of SARS-CoV-2. Set 2 provides detailed guidance on the extraction of RNA from human alveolar-like organoids and the subsequent qPCR to quantify the expression level of e.g., *ACE2* or other host factors of SARS-CoV-2 on RNA level. Protocol set 3 contains an in-depth explanation on how to infect human alveolar-like organoids with SARS-CoV-2 and how to quantify the viral replication by plaque assay and viral E gene-based RT-qPCR. Set 4 provides a step-by-step protocol for the isolation of single cells from infected human alveolar-like organoids for further processing in single-cell RNA sequencing or flow cytometry. Set 5 presents a detailed protocol on how to perform the fixation of human alveolar-like organoids and guides through all steps of immunohistochemistry and *in situ* hybridization to visualize SARS-CoV-2 and its host factors. The infection and all subsequent analytical methods have been successfully validated by biological replications with human alveolar-like organoids based on material from different donors.

COLLECTION INFO

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KEYWORDS

Human Lung Organoids, Protein Extraction, Western Blot, RNA Extraction, RT-qPCR, SARS-CoV-2. Infection, Plaque Assay, viral qPCR, Single Cell Isolation, Single Cell RNA Sequencing, Immunohistochemistry, in situ Hybridization

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GUIDELINES

This protocol collection describes the processing of human alveolar-like organoids which have been grown according to Youk et al., 2020.

<https://doi.org/10.1016/j.stem.2020.10.004>.

BEFORE START

Grow the virus stock (SARS-CoV-2 B.1) on Vero E6 cells (RRID:CVCL_0574), please work with maximum passage 3 and sequence the virus stock initially.

SAFETY WARNINGS

SARS-CoV-2 virus and infected material has to be handled on biosafety level 3 (BSL3).

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Organ models have received widespread attention in the study of SARS-CoV-2, the pathogen causing the current COVID-19 pandemic. Human-based organ models can provide strong predictive value to investigate the tropism, virulence, and replication kinetics of viral pathogens.

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FILES

-   Protein Extraction and Western Blot of Human Lung Organoids
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by **maren.huelsemann**
-   RNA Extraction and RT-qPCR of Human Lung Organoids
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by **morris.baumgardt**
-   SARS-CoV-2 Infection and Viral Replication of Human Lung Organoids
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-   Single Cell Isolation of Human Lung Organoids
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-   Fixation, Immunohistochemistry and in situ Hybridization of Human Lung Organoids
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