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DENGUE Antiviral Immunofluorescence staining Protocol

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

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Abstract

This protocol describes immunofluorescence staining of fixed SARS-CoV-2 infected cells using IC7C7 primary antibody (produced in house against SARS-CoV-2 NP) and Alexa Fluor 488-conjugated secondary antibody. The procedure results in fluorescently labeled infected cells for quantification of viral infection.

Protocol materials

- 0.1% Triton X-100-containing 1XPBS solution
- Tween 20 **Bio-Rad Laboratories Catalog #**170-6606-MSDS
- Goat anti-Rabbit IgG (H L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 **Thermo Fisher Scientific Catalog #**A-11008

Safety warnings

Please be sure to wear proper Personal Protective Equipment (PPE) while performing this experiment.

Before start

Assay is performed under biosafety level 3 conditions.

Post-fix prep

1 Once you have the cells fixed with 10% PFA for minimum 24 hours you may handle the plates.

2 Remove PFA from the wells and discard in the fume hood.

3 Wash the wells 3x with 100ul PBS.

Blocking

4 Add 100ul 1% PBS-BSA to the wells

5 Incubate for 1 hour at RT, then remove

Permeabilization

6 Treat the wells with 100ul 0.1% Triton X-100-containing 1XPBS solution for 10-15 minutes.

7 Remove the triton, and wash 3x with 100ul 0.1% Tween 20 **Bio-Rad Laboratories Catalog #**170-6606-MSDS

Primary Antibody

8 Treat the wells with 50 ul primary flavivirus envelope protein antibody 4G2 (generated in house by Andrew.Duty@mssm.edu) at 0.001mg/ml (**1:1000**) in 1% BSA

Incubate overnight at 4C. If urgent, incubate for 1-2 hours at RT

9 After incubation, wash 2x with 100ul 0.1% Tween 20 **Bio-Rad Laboratories Catalog #**170-6606-MSDS

Secondary Antibody

10 Treat the wells with 50ul secondary antibody

Goat anti-Rabbit IgG (H L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 **Thermo Fisher Scientific Catalog #**A-11008

(1:1000) **and DAPI** in 1% BSA

Incubate overnight at 4C. If urgent, incubate 1-2 hours at RT

11 After incubation, wash 2x with 100ul 0.1%

Tween 20 **Bio-Rad Laboratories Catalog #**170-6606-MSDS

12 Add 100ul plain PBS to the wells for reading and storage