Immunofluorescence assay for detection of ZIKA virus envelope protein

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Abstract

Citation: Elen Mello de Souza,Audrien Alves Andrade,Pedro Paulo de Abreu Manso,Fernanda Cunha Jácome,Marcos Alexandre Nunes da Silva,Gabriela Cardoso Caldas,Ortrud Monika Barth,Debora Ferreira Barreto-Vieira Immunofluorescence assay for detection of ZIKA virus envelope protein. **protocols.io**

dx.doi.org/10.17504/protocols.io.jqycmxw

Published: 05 Sep 2017

Protocol

Step 1.

Seed Vero cells on coverslips into 24- well plate at a density of 2 x 10^5 cells/ well.

Step 2.

Infect with 50 µl of the viral suspension at the multiplicity of infection (MOI) 1 for 1h / 37°C to allow for virus adsorption, under agitation every 15 minutes.

Step 3.

Wash 3 times the cell monolayer with Phosphate-buffered saline (PBSx1) to remove non-adsorbed viral particles.

Step 4.

Follow the infection up to 72 hours.

Step 5.

Each 24 h remove two coverslips out the plate.

Step 6.

Fixation with 500 µl of the 4% paraformaldehyde (PFA) for 20 minutes at 4°C.

Step 7.

Wash 3 times the coverslips with PBS.

Step 8.

Permeabilize the cells with 200 µl of the 0.1% Triton X-100 diluted in PBS for 5 minutes at room temperature.

Step 9.

Wash 3 times the coverslips with PBS.

Step 10.

Block no-specificity binding with 200 µl of the 3% bovine serum albumin (Sigma-Aldrich) for 30 minutes at room temperature.

Step 11.

Wash 3 times the coverslips with PBS.

Step 12.

Incubate with 50 µl of the mouse IgG2a monoclonal antibody anti-ZIKA virus envelope protein (4G2) diluted in PBS (1: 4) for 60 minutes at 37°C.

Step 13.

Wash 3 times the coverslips with PBS.

Step 14.

Incubate with 50 µl of the secondary antibody Alexa Fluor 488 (1: 500) (Invitrogen) for 60 minutes at 37°C.

Step 15.

Wash 3 times the coverslips with PBS.

Step 16.

Incubate with 50 µl of the 10 µg/ ml 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) diluted in PBS for 5 minutes at 37°C, for visualization of the cell nucleus.

Step 17.

Mount the coverslips with 10 µl of the 2.5% 1,4-diazabicyclo(2,2,2)-octane (DABCO) (Sigma-Aldrich) to prevent loss of fluorescence.