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Immunofluorescence Staining Protocol for CMV, YFV and EBOV Antigens

Protocol

Fixation:

Incubate cells with 4% formaldehyde in PBS for 30 mins at room temperature (RT).

Remove formaldehyde and wash once with PBS.

Quench formaldehyde with 50mM Ammonium Chloride/PBS for 20 mins at RT.

Remove Ammonium Chloride and wash once with PBS.

Permeabilization:

Incubate cells with 0.1% Triton X100 in 0.5% BSA/PBS for 10 mins.

Primary antibody staining:

Remove Triton and replace with primary antibody diluted 1:500 (or 1:1000, depending on antibody used) in 0.5% BSA/PBS for 90 mins.

Remove primary antibody and rinse 3 times with 0.5% BSA/PBS.

Secondary antibody staining:

Replace with secondary antibody as per manufacturer's instructions, diluted in 0.5% BSA/PBS, for 60 mins.

Remove secondary antibody and rinse 3 times with 0.5% BSA/PBS.

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Rinse twice with PBS.

Rinse once briefly in water to remove salts.

Apply 1-2 drops of preferred mounting medium on top of coverslip and seal it on microscope slide to start imaging.

This protocol was developed by Virology Research Services:

http://virologyresearchservices.com/