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Immunofluorescence Assay to detect respiratory syncytial virus

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ABSTRACT

IF Protocol used to detect RSV in nasopharyngeal samples.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Matsuno AK, Gagliardi TB, Paula FE, Luna LKS, Jesus BLS, Stein RT, Aragon DC, Carlotti APCP, Arruda E (2019) Human coronavirus alone or in co-infection with rhinovirus C is a risk factor for severe respiratory disease and admission to the pediatric intensive care unit: A one-year study in Southeast Brazil. PLoS ONE 14(6): e0217744. doi: 10.1371/journal.pone.0217744

1 Fix the cells immerge them into a pure cold acetone for 5min.

2 Wash 3X the sample in PBS for 3min each and at room temperature (r.t.).

3 Permeabilize the cells incubate with 0.1% Triton-X100 for 15min at r.t.

4 Wash 3X the sample in PBS for 3min each and at r.t.

5 Incubate the cells with 1:100 dilution of “anti-RSV blend monoclonal antibody” (MAB858-4; Millipore, MA, USA) in PBS with 1% Bovine Serum Albumin (BSA) and 0.2% Tween 20. Keep it inside a humidified chamber for 30min at r.t.

6 Wash 3X the sample in PBS for 5min each and at r.t.

7 Incubate the sample for 15min at r.t in n a Block solution (10% of specific serum diluted in PBS 1X).

Wash 3X the sample in PBS for 3min each and at r.t.

8

9 Incubate the cells with the secondary antibody solution for 30min at r.t. and inside a humidified chamber. It was used “Donkey anti-mouse IgG (H+L) secondary antibody conjugated with Alexa Fluor® 488” (Life Technologies, Thermo Scientific, Carlsbad, CA, USA) diluted 1:200 in PBS with 1% BSA.

10 Wash 3X the sample in PBS for 5min each and at r.t.

11 Incubate the cells with 5µg/ml of DAPI solution (diluted in PBS) for 5min at r.t. and inside a humidified chamber.

12 Wash 2X the sample in PBS for 5min each and at r.t.

13 Mount coverslip with a drop of mounting medium (90% glycerol diluted in PBS).

14 Seal coverslip with nail polish to prevent drying and movement under microscope.

15 Store in dark at -20°C or +4°C.