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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.

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- 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).
- 8** Wash 3X the sample in PBS for 3min each and at r.t.
- 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.