Fluorescent Focus Unit Assay using LICOR Imaging System

Version 2

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Abstract

Indirect immunofluorescence infectivity assay for reovirus using LICOR Imaging System

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Guidelines

This assay is standardized to for 96-well plates using reovirus polyclonal antiserum. New batches of polyclonal antiserum or monoclonal antibodies should be tested to determine optimal dilution.

Before start

Plates and reagents:

Corning Black with clear bottom TC treated 96 well plates (Corning #3904)

DRAQ5 #4084 from Cell Signaling

Sapphire 700 #928-40022 from LICOR

Antibody 800 from LICOR (Life Technologies ones don’t work for this)

Protocol

Step 1.
Remove media from wells.

Step 2.
Wash 1x with Phosphate Buffered Saline (PBS).

Step 3.
Add 100 uL ice-cold methanol per well.

Step 4.

Store plate at -20°C for at least 30 min (can remain at -20°C for several weeks).

Step 5.
Remove methanol, allow plate to come to room temperature, and excess ethanol to evaporate.

Step 6.
Wash wells with 150 ul PBS

Step 7.
Add 150 uL Dulbecco's PBS (DPBS) with 0.5% Tween-20 (DPBS-T) to each well.

Step 8.
Remove immediately.

Step 9.

Add 50 uL rabbit anti-reovirus polyclonal antiserum (1:1000 dilution) in DPBS with 1% BSA (DPBS-BSA) to experimental wells.

Add 50 uL DPBS-BSA with no antibody to background control.

Step 10.
Incubate for 1 h at 37°C.

Step 11.
Remove DPBS-BSA.

Step 12.
Wash 3x with DPBS-T for 5 min each wash while shaking.

Step 13.
Add 50 uL of DPBS-BSA to all wells.

Step 14.
Incubate 1 h at 37°C.

Step 15.
Wash plates 3x with DPBS-T for 5 min each wash.

Step 16.

Prepare cell staining solution in DPBS-BSA:

Secondary antibody (e.g. Goat Anti Rabbit LICOR IRDye 800CW) - 1:1000

Draq5 - 1:10,000

Sapphire700 - 1:1000

Note: we have found that we get less background with the LICOR IRDye 800CW secondary antibody than with Goat Anti Rabbit Alexa 790)

Step 17.

Add 50 uL cell staining solution to all wells that were treated with primary.

Add 50 uL of DPBS-BSA with secondary antibody (1:1000) **only** to background control wells.

Step 18.
Remove solution.

Step 19.
Incubate 1 h at 37°C.

Step 20.
Wash 3x with 150 uL DPBS-T.

Step 21.
Add 50 uL of water.

Step 22.

Scan plates on LICOR Odyssey Imaging System.

a. Focus offset = 3.0 (depends on the plates)

b. 700nm Intensity = 6.5

c. 800nm Intensity = 7.5

Step 23.

Plates and reagents:

Corning Black with clear bottom TC treated 96 well plates (Corning #3904)

DRAQ5 #4084 from Cell Signaling

Sapphire 700 #928-40022 from LICORAntibody 800 from LICOR (Life Technologies secondary antibodies give extra background and should be avoided)