VERSION 1

DEC 07, 2017

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kqicvue

External link:
<https://doi.org/10.1371/journal.ppat.1006768>

Protocol Citation: Bernardo Mainou 2017. Fluorescent Focus Unit Assay using LICOR Imaging System. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.kqicvue>

MANUSCRIPT CITATION:
 Berger AK, Yi H, Kearns DB, Mainou BA (2017) Bacteria and bacterial envelope components enhance mammalian reovirus thermostability. PLoS Pathog 13(12): e1006768. doi: [10.1371/journal.ppat.1006768](https://doi.org/10.1371/journal.ppat.1006768)

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Protocol status: Working

Fluorescent Focus Unit Assay using LICOR Imaging System V.1

PLOS Pathogens

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ABSTRACT

Indirect immunofluorescence infectivity assay for reovirus using LICOR Imaging System

ATTACHMENTS

[Licor FFU.docx](#)

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 INSTRUCTIONS

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)

Created: Nov 08, 2017

Last Modified: Mar 15, 2018

PROTOCOL integer ID:
8682

Keywords: Reovirus,
infectivity, FFU, fluorescent
focus unit

- 1 Remove media from wells.
- 2 Wash 1x with Phosphate Buffered Saline (PBS).
- 3 Add 100 uL ice-cold methanol per well.
- 4 Store plate at -20°C for at least 30 min (can remain at -20°C for several weeks).
- 5 Remove methanol, allow plate to come to room temperature, and excess ethanol to evaporate.
- 6 Wash wells with 150 ul PBS
- 7 Add 150 uL Dulbecco's PBS (DPBS) with 0.5% Tween-20 (DPBS-T) to each well.

- 8** Remove immediately.
- 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.
- 10** Incubate for 1 h at 37°C.
- 11** Remove DPBS-BSA.
- 12** Wash 3x with DPBS-T for 5 min each wash while shaking.
- 13** Add 50 uL of DPBS-BSA to all wells.
- 14** Incubate 1 h at 37°C.
- 15** Wash plates 3x with DPBS-T for 5 min each wash.

- 16** Prepare cell staining solution in DPBS-BSA:
- Secondary antibody (e.g. Goat Anti Rabbit Alexa 488) - 1:1000
Draq5 - 1:10,000
Sapphire700 - 1:1000
- 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.
- 18** Remove solution.
- 19** Incubate 1 h at 37°C.
- 20** Wash 3x with 150 uL DPBS-T.
- 21** Add 50 uL of water.
- 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
- 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)