MAY 04, 2020

OPEN ACCESS

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

Protocol Citation: Cody Roberts 2020. EA.hy 926 RhoA Fret with PAsssr in Flow Cytometry. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bfxujpnw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development
We are still developing and optimizing this protocol

Created: May 04, 2020

Last Modified: May 04, 2020

PROTOCOL integer ID:
36564

Keywords: RhoA Sensor, Flow Cytometry

EA.hy 926 RhoA Fret with PAsssr in Flow Cytometry

Cody Roberts¹

¹Brigham Young University

Cody Roberts
Brigham Young University

ABSTRACT

RhoA Activation test with PAsssr and C3

MATERIALS

C3, PAsssr, VEGF, DMEM, 96 well plate (noncell culture), Trypsin

BEFORE START INSTRUCTIONS

Prechill PBS on ice.

1 Split EA.hy 926 RhoA Sensor Cells into each well of a 6 well Plate. (Seeding density is about

300,000 cells and 1.2 million at confluence. Plate around 500,000 to have cells ready to run the next day. (You will want about 75-95% confluence the day of the experiment.)

- 2 Allow Cells to grow to 75%-95% confluence.
- 3 Morning of, change the media to DMEM (Serfurn Free) supplemented with VEGF. I am not sure what the working concentration Jeremy used for Migration, but I would check his notes and use the same concentration. VEGF can be found in the -80. I believe you will want to use **[M] 0.1 Mass / % volume** BSA as well, but I would again check Jeremy's notes on that.
- 4 Day of experiment, treat cells for 4 to 8 hours depending on the conditions you want. PAsssr for CMG2 targeting should be treated at a concentration of **[M] 200 picomolar (pM)** 200pM, but use **[M] 10 nanomolar (nM)** PAsssr at the beginning to test for the effect because it might be a subtle change. C3 (RhoA inhibitor is at 1ug/ml I believe. I would double check that number.) You will want a no treatment well, RhoA inhibition well (C3), and a CMG2 inhibition well (PAsssr). Unlabeled PAsssr can be found in the frosted -20 in a box labeled PA.
- 5 After the treatment times is over, aspirate the media and add 0.5ml of Trypsin to each well.
- 6 Incubate 10 minutes
- 7 **[Icon: 200xg centrifuge symbol] 200 x g, 24°C, 00:10:00** Move trypsin plus cells to 1.5 ml tubes and spin at 200g for 10 minutes.
- 8 Aspirate media and add 200ul of prechilled PBS. Pipet up and down to break up any clumped cells.

- 9 Add the 200 ul of PBS plus cells to a 96 well plate and leave on ice. Samples are ready to be run in flow.