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May 04, 2020

EA.hy 926 RhoA Fret with PAsssr in Flow Cytometry

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In Development dx.doi.org/10.17504/protocols.io.bfxujpnw

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ABSTRACT

RhoA Activation test with PAsssr and C3

MATERIALS TEXT

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

BEFORE STARTING

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 (Serfum Free) supplimented 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 aswell, 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 begining 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). Unlableled PAsssr can be found in the frosted -20 in a box labeled PA.

5 After the treatment times is over, asparate the media and add 0.5ml of Trypsin to each well.

6 Incubate 10 minutes

7 **200 x g, 24°C 00:10:00** Move tyrpsin plus cells to 1.5 ml tubes and spin at 200g for 10 minutes.

8 Asparate media and add 200ul of prechilled PBS. Pipet up and down to break up any clumped cells.

Citation: Cody Roberts (05/04/2020). EA.hy 926 RhoA Fret with PAsssr in Flow Cytometry. https://dx.doi.org/10.17504/protocols.io.bfxujpnw

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.

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