

May 04, 2020

EA.hy 926 RhoA Fret with PAsssr in Flow Cytometry

DOI

<https://dx.doi.org/10.17504/protocols.io.bfxujpnw>

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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>

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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, flow cytometry rhoa activation test with passsr, flow cytometry rhoa activation test, rhoa fret with passsr, flow cytometry, rhoa fret, passsr,

Abstract

RhoA Activation test with PAsssr and C3

Materials

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

Before start

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 (Serfom 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). Unlabeled 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.
- 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.