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Flow cytometry for ex vivo stimulated pMacs

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Rebecca Wallings[1]

[1]University of Florida

Rebecca Wallings
University of Florida

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Protocol status: Working
We use this protocol and it's working

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Abstract

Flow cytometry for ex vivo stimulated pMacs

1 Incubate cells for 30 minutes at 37 degrees in incubator in media containing 3mM EDTA at pH 6.2 to gently lift cells from plate. To encourage dissociation from plate, gently tap plate, or alternatively use a mini scraper

2 Aliquot cells in to wells of v-bottom 96 well plate

Spin cells at 300 x g at 4 degrees for 5 minutes

Resuspend cells in 50uL of antibody cocktail containing live/dead dye and Fc block

Incubate at 4 degrees in the dark for 20 minutes

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 200uL 1 x PBS

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 200uL 1 x PBS

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 50uL ICC fixation buffer (Invitrogen)

Incubate cells at room temperature for 10 minutes in the dark

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 200uL FACS buffer and proceed to assess cells on cytometer