May 03, 2024

Intracellular and extracellular MHC-II immunostaining and microscopy on peritoneal macrophages

DOI
dx.doi.org/10.17504/protocols.io.bp2l62191gqe/v1

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Protocol Citation: Rebecca Wallings 2024. Intracellular and extracellular MHC-II immunostaining and microscopy on peritoneal macrophages. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l62191gqe/v1

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

Created: May 03, 2024

Last Modified: May 03, 2024

Protocol Integer ID: 99196

Abstract

Staining of intracellular and extracellular MHCII in peritoneal macrophages in 96 well plate using EVOS fluorescent microscope and analysis on CellProfiler

1 After harvesting and plating pMacs from mouse, plate in 96-well plate at 50,000 cells/well in 200uL as described in pMac harvesing protocol. Once cells have adhered, aspirate media and replace with fresh growth media containing vehicle or 100U of IFNy for 18

2 Retrieve cells from 37 degrees. Aspirate media and wash 3x DPBS+/+

3 Incubate cells with 25 ug/mL of APC-MHC-II (Biolegend, RRID:AB_313329) in DPBS+/+ containing 1:100 FcR blocking reagent, final volume 50uL, for 30 minutes at room temperature, protected from light

4 Wash cells 3 x DPBS+/+

5 fix cells by incubation in 4% PFA for 10 minutes at room temperature, final volume of PFA 50uL, and then washed 3x with DPBS+/+

6 Permeabilize cells with 100uL permeabilization buffer (eBiosciences, #88-8824-00) on ice for 15 minutes

7 Spike in 25ug/mL of PE-610-MHC-II (Biolegend, RRID:AB_2574618) into permeabilization buffer and incubate cells for 30 minutes at room temperature protected from light.

8 Wash cells 3 x DPBS+/+

9 Incubate cells in 1 µg/ml DAPI (Invitrogen, RRID:AB_2629482) for 10 minutes at room temperature in DPBS+/+, final volume 50uL.

10 Image cells on an EVOS™ M7000 (Invitrogen) at 20 x magnification.

11 Perform image analysis using Cellprofiler 4.2.5 (RRID:SCR_007358). Using the 'IdentifyPrimaryObject' module in Cellprofiler, identify icMHC and exMHC in their respective channels and quantify MFI and calculate Ex:IcMHCII from these quantified MFI values.