May 04, 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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DOI: https://dx.doi.org/10.17504/protocols.io.bp2l62191gqe/v1

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 04, 2024

Protocol Integer ID: 99196

Keywords: extracellular mhcii in peritoneal macrophage, microscopy on peritoneal macrophage, extracellular mhc, extracellular mhcii, peritoneal macrophage, ii immunostaining, using evos fluorescent microscope, evos fluorescent microscope, microscopy

Abstract

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

Troubleshooting

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.