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immunofluorescent staining with anti-GFP and anti-CD63 antibodies

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

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ASAP

Abstract

This protocol was used for immunofluorescent staining in fixed HeLa cells with anti-GFP and anti-CD63 antibodies, followed by confocal imaging.

Protocol materials

FluorSave™ Reagent **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**345789

Troubleshooting

1 plate cells on glass coverslips and grown until they reach 60% conlfuency

2 fix cells with 4% paraformaldehyde for 20 min at room temperature

3 permeabilize cells with 0.1% Triton X-100 in PBS for 5 min

4 block for 1 h with blocking buffer (PBS with 0.5% Tween20, 0.1% BSA, 0.2% FBS)

5 incubate coverslips with primary antibodies for 2 h at room temperature
(anti-CD63, exbio, 11-343-C100, mouse; anti-GFP, abcam, ab13970, chicken)

6 wash coverslips 3 times with PBS-T

7 incubated coverslips 30 min with secondary antibodies
(goat-anti-mouse-AlexaFluor647, goat-anti-chicken-AlexaFluor488)

8 wash coverslips 3 times with PBS-T

9 incubate coverslips with DAPI

10 wash coverslips 3 times with PBS-T

11 mounte coverslips using
FluorSave™ Reagent **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**345789

12 images were acquired using an LSM780 con- focal microscope (Zeiss) with a 10x or 40× objective

13 colocalization analysis was performed with Fiji plugin Jacop