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Lipid droplet visualisation in cultured cells using BODIPY 493/503 stain

Laura Smith[1]

[1]Department of Clinical and Movement Neurosciences, Queen Square Institute of Neurology, University College London (UCL)

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Laura Smith

Lipid droplets are organelles involved in intracellular lipid homeostasis, as well as playing key roles in a variety of cellular functions including cellular signalling, metabolic disease and inflammation. BODIPY™ 493/503 (4,4-Difluoro-1,3,5,7,8-Pentamethyl-4-Bora-3a,4a-Diaza-*s*-Indacene) is a fatty acid-conjugated fluorescent probe that has been shown to successfully visualise the localisation and dynamics of lipid droplets in cells. Analysis can be performed on cells cultured in complete growth medium or in cells following lipid loading with oleic acid (OA) after over-night starvation in OptiMEM. OA is a potent inducer of lipid droplet formation.

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Principle

1 Lipid droplets are organelles involved in intracellular lipid homeostasis, as well as playing key roles in a variety of cellular functions including cellular signalling, metabolic disease and inflammation. BODIPY™ 493/503 (4,4-Difluoro-1,3,5,7,8-Pentamethyl-4-Bora-3a,4a-Diaza-*s*-Indacene) is a fatty acid-conjugated fluorescent probe that has been shown to successfully

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visualise the localisation and dynamics of lipid droplets in cells. Analysis can be performed on cells cultured in complete growth medium or in cells following lipid loading with oleic acid (OA) after over-night starvation in OptiMEM. OA is a potent inducer of lipid droplet formation.

Basal conditions

2 Plate cells onto coverslips in 6 wells plate – want cells to be 60-80% confluent for analysis.

3 Remove media and wash 2x in PBS

4 Fix cells in 4% PFA at RT for 20 minutes.

5 Wash 1x in PBS

6 Make up BODIPY 493/503:
- Stock is 1mg/ml in DMSO
- Keep away from light (always in foil)

7 Dilute BODIPY 493/503 in PBS at 1/1000 dilution
- Example: if staining 21 coverslips = 250 x 21 = 5,250 uL
- 5.5 uL BODIPY stock in 5,500 uL PBS

8 Add 250 uL BODIPY per coverslip

9 Incubate coverslips at room temperature for 15 minutes (keep away from light)

10 Wash coverslips 3x in PBS

11 Mount coverslips with DAPI+Citifluor and analysed by fluorescence microscopy.

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Lipid loading conditions

12 Plate cells onto coverslips in 6 wells plate – want cells to be 60-80% confluent for analysis.

13 Remove media from cells and replace with OptiMEM overnight

14 Make up 10 mM oleic acid (1 uL OA in 170uL PBS/BSA)

15 Dilute oleic 1/100 in OptiMEM for 5 hours in incubator
- Example: If 1.5 mL optiMEM in well add 15 uL OA to the well

16 Fix cells in 4% PFA at RT for 20 minutes.

17 Wash 1x in PBS

18 Make up BODIPY 493/503:
- Stock is 1mg/ml in DMSO
- Keep away from light (always in foil)

19 Dilute BODIPY 493/503 in PBS at 1/1000 dilution
- Example: if staining 21 coverslips = 250 x 21 = 5,250 uL
- 5.5 uL BODIPY stock in 5,500 uL PBS

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22 Wash coverslips 3x in PBS

23 Mount coverslips with DAPI+Citifluor and analysed by fluorescence microscopy.

Counting lipid droplets

24 To count lipid droplets, the ImageJ software was used. The local intensity maxima of each lipid droplet was determined and counted in pre-processed images.

24.1 Convert the image to a grayscale 8-bit image.

Image > Split channels (work on the image with the lipid droplets not DAPI)
Image > Type > 8-bit (repeat 2x)

24.2 To ease localisation and separation of lipid droplets, subtract the background from the image.

Process > Subtract background – change the number to the best number to not subtract any lipid droplets (my image was 25)

24.3 Use the find maxima tool to count each lipid droplet in the picture.

Process > Find Maxima
Select count and click preview point selection to visualise each dot that will be measured.
Adjust noise tolerance – the higher the number the more specific so fewer dots will be selected. Want to play around with this until you get a number that is picking up most of the dots with minimal background (my image was around 15-18)
Number appears at bottom of window as x Maxima.

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