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Fatty Acid Induction to Hippocampal Neurons

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We use this protocol and it's working

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Abstract

This protocol provides a stepwise procedure for preparing homogeneous fatty acid–BSA complexes for lipid droplet induction in primary cultured hippocampal neurons. The protocol also describes the fatty acid induction to primary hippocampal neurons for electrical stimulation experiments.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

Materials:

- Oleic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**O1383
- Palmitic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P0500
- BSA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**#A8806
- Tris Buffer: 0.1 M Tris (pH 7.4 using NaOH)
- NaOH Solution: 0.1 M NaOH (200 mg NaOH in 50 ml ultrapure water)
- NaCl Solution: 0.9% NaCl (450 mg NaCl in 50 ml ultrapure water)
- BODIPY™ 493/503 (4,4-Difluoro-1,3,5,7,8-Pentamethyl-4-Bora-3a,4a-Diaza-s-Indacene) **Thermo Fisher Catalog #**D3922

Equipments:

- Heating block (temperature controlled to 40°C and 70°C as required)
- Syringe filter: 0.22 µm pore size
- Culture hood
- Aluminum foil
- Ultrapure water
- Vortex heater

Procedure to prepare Oleic Acid–BSA (OA-BSA) Complex

1d 3h 10m

1 **Prepare 0.1 M Tris Buffer (pH 7.4):**

Dissolve Trizma in ultrapure water and adjust pH to 7.4 using NaOH.

2 **Dissolve BSA:**

2.1 Pour 4 mL of [M] 0.1 Mass Percent Tris into a 50 ml tube. Overlay 697.5 mg of FA-free BSA on top.

2.2 Place the tube on a heating block maintained at 42 °C and stir gently until the BSA is completely dissolved. Adjust the final volume to 4.98 mL with [M] 0.1 Mass Percent Tris buffer.

3 **Add Oleic Acid:**

Thaw OA at 37 °C. Add 19.83 µL of OA to the BSA solution.

4 **Incubate:** 3h

Wrap the tube with aluminum foil to protect from light. Incubate at 37 °C with gentle shaking for 02:00:00 - 03:00:00. Ensure that the solution becomes homogeneously clear (turbidity should disappear).

5 **Filter the Complex:**

Filter the solution through a 0.22 µm syringe filter in a sterile culture hood. Aliquot 250 µL portions and store at 4 °C.

6 **Induction of Lipid droplets:** 1d

Add OA-BSA complex to 14-21 DIV culture primary hippocampal neurons/fibroblasts to the final concentration of 200 µL - 400 µL for 24:00:00.

7 **Detection of lipid droplets:**

10m

Add 1 µL BODIPY to the culture media and return the dishes to incubator for 00:10:00. Wash the cells with PBS and mount the glass coverslip to image on confocal microscope.

Procedure to prepare Palmitic Acid–BSA (PA-BSA) Complex

4h 20m

8 **Prepare Solutions:**

8.1 Prepare [M] 0.1 Mass Percent NaOH by dissolving 200 mg NaOH in 50 mL ultrapure water.

8.2 Prepare 0.9% NaCl Solution by dissolving 450 mg NaCl in 50 mL ultrapure water.

9 **Dissolve BSA:**

9.1 Pour 3.5 mL of 0.9% NaCl solution into a 50 ml tube. Overlay 555.55 mg of FA-free BSA on top.

9.2 Place the tube on a heating block maintained at 40 °C and stir gently until the BSA is completely dissolved.

9.3 Adjust the final volume to 4 mL with 0.9% NaCl solution. Aliquot 800 µL portions into 5 Eppendorf tubes and maintain at 42 °C on a vortex heater.

10 **Dissolve Palmitic Acid:**

Weigh 15.38 mg of PA and dissolve in 1 mL of [M] 0.1 Mass Percent NaOH. Heat the mixture to 70 °C on a heating block, carefully monitoring the temperature to prevent overheating.

11 **Complexing PA with BSA:**

Immediately add 200 µL of the PA solution to each 800 µL aliquot of BSA maintained at 40 °C while vortexing.

Note

PA may solidify if the temperature drops.

12 **Incubate:**

20m

Leave PA-BSA solution under agitation for 00:15:00 - 00:20:00 . The solution may appear turbid/whitish at this stage.

13 **Filter the Complex:**

Filter the solution through a 0.22 µm syringe filter in a sterile culture hood. Prepare the complex fresh before use and maintain the temperature at 37 °C until used.

14 **Induction of neurons:**

4h

Add PA-BSA complex to the media of primary hippocampal neurons to the final concentration of [M] 150 micromolar (µM) for 04:00:00 .

Note

- Protect fatty acid solutions from light during incubation to prevent photooxidation.
- Ensure precise temperature control during the preparation of both complexes to avoid solidification or denaturation.
- Always use sterile techniques and equipment to prevent contamination.