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Complexing Sodium Oleate for use in insulin secretion

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1 Works for me dx.doi.org/10.17504/protocols.io.buamnsc6

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ABSTRACT

This protocol is for complexing of sodium oleate to be used in insulin secretion experiments.

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MATERIALS TEXT

BSA (Essentially Fatty acid free): Sigma A6003
Sodium Oleate: Sigma O3880

Preparation of 10% w/v BSA

1 Weigh **1 g** of fatty acid free BSA (Sigma A6003)

2 Add BSA into a beaker with **10 mL** of ultrapure water

3 Stir BSA and water until all the BSA has dissolved.

*Warming at **37 °C** may help dissolve BSA.

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4 Filter BSA solution with 0.22µM filter. This can be made in advance and stored at **4 °C** .

Preparing 150mM stock of sodium oleate

5 Weight approximately **45.7 mg** of sodium oleate (MW 305g/L).

*It should be noted that sodium oleate is very hydrostatic. Weigh approximately the amount indicated above, then calculate the volume of solvent needed to create a 150mM solution. 50% of this volume is then added in step 6, and 50% in step 8.

*This must be made fresh the day of your experiment.

6 Add **500 µl** (or as calculated) of 100% Ethanol and vortex

7 Using a heat block, heat at **65 °C** for **00:15:00** 15m

8 After 15 minutes the sodium oleate should be solubilized. Add **500 µl** (or as calculated) of ultrapure water. No remaining undissolved solute should be visible at this time.

9 Vortex and continue to heat solution at **65 °C** for **00:10:00** . 10m

10 Vortex and hold the sodium oleate at **65 °C** until complexed with BSA.

Complexing oleate with BSA (5:1) 1h 10m

11 *Below numbers are based on **10 mL** of solution. Adjust volume as necessary for each experiment* 5m

Warm 2 tubes of **670 µl** of 10% BSA for **00:05:00** in a **37 °C** water bath.

One tube will be for your control group. One group for your oleate group.

12 For control group, add **33 µl** of 50% ethanol to one pre-warmed BSA tube from step 11.

13 For oleate group add **33 µl** of **150 Milimolar (mM)** oleate stock to one pre-warmed BSA tube from step 11.

Important note It is very important to keep BSA warm when adding oleate. If hot plate and water bath are not beside each other, place tube with BSA in a beaker containing 37°C of water.

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1h 5m

14 Incubate both control and oleate tubes in at shaking water bath (**37 °C**) for **01:00:00** .

*Check oleate+BSA tube after approximately **00:05:00** to make sure the solution isn't cloudy. If it is cloudy, discard and restart at step 11.

*If you are using KRBH for your secretion experiment, make sure it is prepared prior to the end of this hour.

15 After 1 hour add **9.3 mL** of warm KRBH (or solution to be used for experiment) to each tube. Add glucose or other compounds as needed. Solution must be kept at **37 °C** and used on the same day it is made.

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