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# Preparation of BSA complexed free fatty acids for in vitro studies

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This protocol describes the steps to prepare a fatty acid:BSA complex in cell culture media. It is suitable for the treatment of primary tissue (eg. isolated islets) and cell lines in ex vivo culture. Briefly, fatty acid stock solutions are prepared and complexed with BSA prior to dilution in culture media. Final fatty acid concentrations range from 0.125 to 0.5 mM. We recommend replacing the fatty acid mixture once each day throughout the treatment.

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Briaud I, Harmon JS, Kelpe CL, Segu VB, Poitout V. Lipotoxicity of the pancreatic beta-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. Diabetes. 2001 Feb;50(2):315-21. doi:10.2337/diabetes.50.2.315. PMID: 11272142.

fatty acids, BSA complex, cell culture

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MilliQ Water **Contributed by users**

Ethanol, Anhydrous **Bio Basic Inc. Catalog #D0193**

Steriflip-GP Sterile Centrifuge Tube Top Filter Unit **Millipore Catalog #SCGP00525**

sodium oleate **Millipore Sigma Catalog #O7501**

Sodium palmitate **Millipore Sigma Catalog #P9767**

Fatty Acid Free Heat Shock Bovine Serum Albumin Powder **equitech bio, inc. Catalog #BAH66-0500**

## Preparation of BSA:FA complex 1h 25m

### 1 Preparation of Palmitate (PA) and Oleate (OA) stock solutions 15m

As PA and OA are in powder form and very hydrostatic, handle with care as the powder will stick to everything and move about readily.

Carefully weigh out a sufficient amount of each to make **150 millimolar (mM)** stock solutions in ethanol.

MW (PA) = 278.41, stored in a desiccator at **4 °C** .

MW (OA) = 305, stored in a desiccator at **-20 °C** .

Preparation of **1 mL** of **150 millimolar (mM)** stock solution:

**PA** Weight out **41.8 mg** and put in a 1.5 ml tube.

Add **1 mL** of **50 % (v/v)** ethanol.

Vortex and heat at **65 °C** until dissolved; vortex periodically.

**OA** Weight out **45.7 mg** and put in a 1.5 ml tube.

Add **500 µL** of **100 % (v/v)** ethanol.

Vortex and heat at **65 °C** for **00:15:00** ; mix occasionally.

Then add **500 µL** of MilliQ water, and vortex. The remaining powder should dissolve instantly.

Return to **65 °C** until ready for use.



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## 2 Preparation of 0.5 mM fatty acid solution in culture media (FA:BSA at 5:1 molar ratio) h 10m

Prepare [M]**10 % (v/v)** fatty acid-free BSA:

Weight **5 g** and dissolve in **50 mL** of MilliQ water.

Filter solution with a Millipore Steriflip-GP Sterile Centrifuge Tube Top Filter Unit.

Store at **4 °C** .

Under a laminar flow hood, add the following components per ml of culture media:

Place **67 µL** of [M]**10 % (v/v)** fatty acid-free BSA into a 15 ml Falcon tube.

Heat in **37 °C** water bath for **00:05:00** .

Add **3.3 µL** of the [M]**150 millimolar (mM)** fatty acid stock solution.

Return to the **37 °C** water bath for **00:05:00** . Visually check for cloudiness. If clear, great, if cloudy, start over.

Incubate in the **37 °C** water bath for **01:00:00** .

Then add **930 µL** of culture medium (eg. RPMI 1640 plus [M]**10 % (v/v)** FBS) heated to **37 °C** .

For control tube (Vehicle/BSA), add **3.3 µL** of [M]**50 % (v/v)** ethanol to **67 µL** of [M]**10 % (v/v)** fatty acid-free BSA into a 15 ml Falcon tube, and treat as above.

## 3 Preparation of 0.15 mM fatty acid solution in culture media (FA:BSA at 1.5:1 molar ratio)

To prepare a solution with a final fatty acid concentration of [M]**0.125 millimolar (mM)** proceed as described in step 2 adding **1 µL** of the [M]**150 millimolar (mM)** fatty acid stock solution (instead of **3.3 µL** ). Also reduce the ethanol to **1 µL** for the control.

To prepare any fatty acid concentration up to [M]**0.5 millimolar (mM)** final adjust the amount of fatty acid stock solution accordingly while keeping the BSA constant.



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