Pancreatic Insulin Content by Acid-Ethanol Extraction

Version: 1
Replaced by version: N/A
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Summary: Used to calculate the insulin content in the pancreas.

Protocol:

1) ¼ - ½ of the pancreas is placed into 5 ml Acid-Ethanol (1.5% HCl in 70% EtOH) in a 15 ml conical vial.

2) Incubate O/N at -20°C.

3) Homogenize tissue (I use a Polytron homogenizer).

4) Incubate O/N at -20°C.

5) Centrifuge at 2000 rpm 15 min at 4°C (Sorvall RT6000).

6) Transfer aqueous solution to a new 15 ml conical vial.

7) Neutralize 100 µl of Acid-Ethanol extract with 100 µl 1M Tris pH 7.5.

8) Dilute further (1:100, 1:1000, or 1:5000 depending upon the strain) in Insulin ELISA sample diluent.

9) Run diluted sample on Insulin ELISA (Exocell). Calculate ng/ml with appropriate dilution factor.

10) Run 20 µl of the neutralized solution in a Bradford Assay (250 µl Coomassie Blue Reagent, Thermo Scientific) against a standard curve. Calculate µg/ml with appropriate dilution factor.

11) Divide Insulin content ng/ml by Protein content µg/ml.