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Pancreatic Insulin Content by Acid-Ethanol Extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.3jjgkkn

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ABSTRACT

Summary:

Used to calculate the insulin content in the pancreas.

Diabetic Complications:

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=73&docType=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.