ABSTRACT

Summary:

Used to calculate the insulin content in the pancreas.

Diabetic Complications:

- Cardiovascular
- Nephropathy
- Neuropathy

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Protocol status: Working

We use this protocol and it's working
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 -20ºC.

3 Homogenize tissue (I use a Polytron homogenizer).
4 Incubate O/N at -20ºC.

5 Centrifuge at 2000 rpm 15 min at 4ºC (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.