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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](https://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:



Cardiovascular



Nephropathy



Neuropathy



Pediatric Endocrinology



Retinopathy



Uropathy



Wound-Healing

### EXTERNAL LINK

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

- 1  $\frac{1}{4}$  -  $\frac{1}{2}$  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  $\mu$ l of Acid-Ethanol extract with 100  $\mu$ l 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  $\mu$ l of the neutralized solution in a Bradford Assay (250  $\mu$ l Coomassie Blue Reagent, Thermo Scientific) against a standard curve. Calculate  $\mu$ g/ml with appropriate dilution factor.
- 11 Divide Insulin content ng/ml by Protein content  $\mu$ g/ml.



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