AUG 12, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3jjgkkn

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

Protocol Citation: Ed Leiter 2019. Pancreatic Insulin Content by Acid-Ethanol Extraction. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3jjgkkn>

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Protocol status: Working
 We use this protocol and it's working

Created: May 31, 2019

Last Modified: Aug 12, 2019

PROTOCOL integer ID:
 23883

🌐 Pancreatic Insulin Content by Acid-Ethanol Extraction

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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

Keywords: Pancreatic Insulin,
Acid-Ethanol Extraction,
cardiovascular, nephropathy,
neuropathy, pediatric
endocrinology, retinopathy,
uropathy, wound-healing

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