Aug 12, 2019

Pancreatic Insulin Content by Acid-Ethanol Extraction

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

Ed Leiter[1]

[1]Jackson Laboratories

Diabetic Complications Consortium
Tech. support email: **rmcindoe@augusta.edu**


Lili Liang

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

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

Created: May 31, 2019

Last Modified: August 12, 2019

Protocol Integer ID: 23883

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

Abstract

Summary:

Used to calculate the insulin content in the pancreas.

Diabetic Complications:

Cardiovascular

Nephropathy

Neuropathy

Pediatric Endocrinology

Retinopathy

Uropathy

Wound-Healing

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.