

# Analysis of insulin, C-peptide or proinsulin from acid ethanol extractions from islets, cells or tissue

To measure insulin, C-peptide or proinsulin content from islets, cell-lines or pancreas, hormones may be extracted from tissues/cells using ethanol extraction. Extractions can then be diluted and analyzed in MercoDia Insulin, C-peptide or Proinsulin ELISAs.

## Acid ethanol extraction

For islets or cells, it is recommended to sonicate islets or cells in water (for example 0.1 mL water to 20 islets, approximately 40.000 cells, sonication 15 seconds). The sonicate is mixed with acid ethanol (0.18 M HCl in 96% ethanol (vol/vol)), in a 1:3 proportion of sonicate and acid ethanol.

The mixed solution is incubated in 4°C for 12 hours. (This extracts the insulin, C-peptide and proinsulin). Detailed protocols are found in the references below. The acid ethanol extracts may be stored in -70°C until dilution and analysis in ELISA.

For whole pancreas extraction, it is recommended to remove 10-20 mg of pancreas and sonicate in acid ethanol (note that 70% ethanol is used for whole pancreas compared to 96% for islets or cells) 0.18 M HCl in 70% (vol/vol) ethanol.

Put the sonicate in 4°C for 12 hours. (This extracts the insulin, C-peptide and proinsulin). Detailed protocols are found in the references below. The acid ethanol extracts may be stored in -70°C until dilution and analysis in ELISA.

## Analysis in MercoDia ELISA

Before analysis in MercoDia Insulin, C-peptide or Proinsulin ELISA assays, it is important that samples are diluted at least 100 times to ensure that the acid ethanol do not interfere in the ELISA procedure. Vortex samples, centrifuge to avoid adding cellular debris, dilute at least 100 times in Calibrator 0 or in MercoDia Diabetes Sample Buffer. Add diluted sample as described in MercoDia ELISA directions for use.

**References**

Andersson AK and Sandler S (2001) Melatonin protects against streptozotocin, but not interleukin-1beta-induced damage of rodent pancreatic beta-cells. *J Pineal Res.* 2001 30(3):157-65.

Carlsson A, Hallgren IB, Johansson H and Sandler S (2010) Concomitant enzyme-linked immunosorbent assay measurements of rat insulin, rat C-peptide, and rat proinsulin from rat pancreatic islets: effects of prolonged exposure to different glucose concentrations. *Endocrinology* 151(10):5048-52.