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Preparation of Pancreas Lysate

1. Pancreas organs were removed from the animal and flash frozen in liquid nitrogen. Organs were kept at 80ºC until use.
2. The frozen tissue was suspended in 5 volumes of ice-cold lysis buffer containing 50mM Tris (ph 7.4), 5mM EDTA (ph 8), 1% Triton X-100, and protease inhibitor cocktail (Complete, EDTA-free, Roche).
3. The tissue was immediately homogenized with a Polytron homogenizer. Homogenates were then centrifuged at 150,000 *g* for 30 minutes at 4ºC.
4. The resulting supernatant was transferred to a clean tube and protein content was established. Protein concentration was adjusted to 3 mg/ml with lysis buffer. The lysates were kept at -80ºC until use.