insane: INsulin Secretion ANalysEr

Amélie Bonnefond, Ph.D.

### Experimental Protocol

#### Culture of EndoC-βH1 cells

EndoC-βH1 cells are cultured in Optiβ medium (Univercell). Cells are seeded at a density of 2.5 × 106 in T25 flasks coated with β-coat (Univercell) according to manufacturer’s instructions, and cultured at 37 °C and 5 % CO2.

#### Transfection of siRNA into EndoC-βH1 cells

In case of transfection, siRNAs are transfected into EndoC-βH1 cells using Lipofectamine 2000 (Invitrogen).  
Freshly trypsinized EndoC-βH1 (0.5 × 106 cells) are incubated in suspension with Lipofectamine-siRNA complex in Opti-MEM (Invitrogen) containing 50 μM 2-mercaptoethanol, 10 mM nicotinamide (Calbiochem, Merck Millipore), 5.5 mg/ml human transferrin (Sigma-Aldrich), 6.7 ng/ml sodium selenite (Sigma-Aldrich), 100 U/ml penicillin and 100 mg/ml streptomycin (Life Technologies) for 10 minutes and then are plated onto culture wells coated with β-oat (Univercell).  
Six hours later, the medium is replaced.  
We previously used ON-TARGETplus siRNA SMARTpool for each gene of interest (20-30 nM) and ON-TARGETplus nontargeting pool for controls (siNTP; Dharmacon, Thermo Fisher Scientific, Waltham, MA, USA) (Ndiaye et al. [2017](#ref-ndiaye_expression_2017)).  
Cells are analyzed 72 hours post transfection.

#### Assessment of insulin secretion from EndoC-βH1 cells

EndoC-βH1 cells seeded onto β-coat-coated 96-well plates at 5 × 104 cells/well are incubated overnight in no glucose Dulbecco’s Modified Eagle’s Medium (Sigma Aldrich) supplemented with 2 % BSA Fraction V, 50 μM 2-mercaptoethanol, 10 mM nicotinamide (Calbiochem, Merck Millipore), 5.5 mg/ml human transferrin (Sigma-Aldrich), 6.7 ng/ml sodium selenite (Sigma-Aldrich), 100 U/ml penicillin and 100 mg/ml streptomycin (Life Technologies) and that contained 2.8 mM glucose.  
The day after, they are incubated in HEPES-buffered Krebs-Ringer Buffer (KRB; 116 mmol/l NaCl, 5.06 mmol/l KCl, 1.007 mmol/l CaCl2, 1.01 mmol/l MgCl2, 23.96 mmol/l NaHCO3, 10 mmol/l HEPES, pH 7.4, and 0.1 % BSA solution) that contained 0.5 mM glucose ± 0.5 mM IBMX (Sigma Aldrich) for 60 minutes at 37 °C.  
This supernatant is subsequently collected (supernatant 1 [SN1]) and replaced with 16.7 mM glucose KRB ± 0.5 mM IBMX for a 60-minute incubation at 37 °C and then collected (supernatant 2 [SN2]).  
For insulin content measurement, cells are lysed with TETG buffer (20 mM Tris-HCl pH 8.0, 137 mM NaCl, 1 % Triton X-100, 10 % Glycerol, 2 mM EGTA with protease inhibitors; Roche).  
Lysate, SN1 and SN2 are centrifuged for 5 minutes at 700 g.  
Samples are kept frozen at -20 °C before use.  
Insulin concentration in the supernatants and intracellular content of the EndoC-βH1 cells are measured by ELISA according to manufacturer’s instructions using the Human Insulin Kit (Mercodia, Uppsala, Sweden).  
Briefly, 25 µl of either blank, standard (from 0.2 to 6 µg/ml), or diluted samples (1:400 for lysates and 1:16 for supernatants) and 100 µl 1X conjugate enzyme are put in the 96-well plate coated with insulin antibody for 1 hour under agitation at room temperature.  
The plate is then washed six times with wash buffer 1X solution.  
Then, 200 µl substrate is added in the plate for a 15-minute incubation.  
Then, 50 µl stop solution is added and the absorbance at 450 nm is measured, using technical duplicates of experimental triplicates, leading to six measurements per experimental conditions (that are subsequently repeated).

*Note*: absorbance values need to be written within the Excel template provided by the **insane** package.

#### References

Ndiaye, Fatou K., Ana Ortalli, Mickaël Canouil, Marlène Huyvaert, Clara Salazar-Cardozo, Cécile Lecoeur, Marie Verbanck, et al. 2017. “Expression and Functional Assessment of Candidate Type 2 Diabetes Susceptibility Genes Identify Four New Genes Contributing to Human Insulin Secretion.” *Molecular Metabolism* 6 (6): 459–70. <https://doi.org/10.1016/j.molmet.2017.03.011>.