Measurement of insulin concentration V.3

PeerJ

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Dec 11, 2019

1 Works for me

dx.doi.org/10.17504/protocols.io.v63e9gn

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EXTERNAL LINK

https://doi.org/10.7717/peerj.8157

BEFORE STARTING

Insulin secreted into the culture medium from MIN6 and INS-1 cells was measured using the Mouse and Rat Insulin H-type™ enzyme-linked immunosorbent assay kit (Shibayagi Co. Ltd., Shibukawa, Japan), according to the manufacturer's protocol (Suzuki K. et al. 2015, PeerJ 3:e1498. doi: 10.7717/peerj.1498).

1 MIN6 cells were cultured for 30 min in Krebs-Ringer bicarbonate HEPES (KRBH) buffer (140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 2 mM $NaHCO_3$, 10 mM HEPES, and 0.1% bovine serum albumin) containing 2 mM glucose.

2 The buffer was then changed to another KRBH buffer solution containing the relevant experimental concentration of glucose, and the cells were cultured for 1 h.

3 The KRBH buffer was collected and its insulin concentration was measured.

4 The insulin concentration of the buffer was normalized to the total cell protein level.

The results were normalized to the concentration of control samples of each independent experiment and the normalized values were shown as insulin secretion ratio.