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ADP assay (Colorimetric)

PeerJ

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1 Works for me dx.doi.org/10.17504/protocols.io.zk4f4yw

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EXTERNAL LINK

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kusunoki M, Hayashi M, Shoji T, Uba T, Tanaka H, Sumi C, Matsuo Y, Hirota K, Propofol inhibits stromatoxin-1-sensitive voltage-dependent K channels in pancreatic β-cells and enhances insulin secretion. PeerJ doi: 10.7717/peerj.8157

MATERIALS

NAME	CATALOG #	VENDOR
ADP Assay Kit (Colorimetric/Fluorometric)	ab83359	Abcam

Standard preparation

1 Reconstitute the ADP standard (1 µmol) in 100 µl of water to generate a 10 mM ADP stock solution.

2 Dilute 10 µl of the 10 mM ADP stock solution with 90 µl of ADP Assay Buffer to prepare a 1 mM ADP standard solution.

3 Prepare dilutions of a ADP standard using ADP Assay Buffer (0, 0.04, 0.08, 0.12, 0.16, 0.2 mM). 100 µl of each dilution is required to set up duplicate reading (2 x 50 µl).

Sample preparation

4 Prepare cells in a 12-well plate.

5 Remove media and wash cells with cold PBS.

6 Lyse cells in 100 µl of ADP Assay Buffer.

7 Incubate the lysate for 10 min on ice.

8 Centrifuge at 18,000 x *g* for 5 minutes at 4°C.

9 Collect the supernatant in a new microcentrifuge tube and keep on ice.

Assay procedure (for the colorimetric assay)

10 Test all samples with a minimum of two replicates.

11 Dilute the lysate 3-fold in ADP Assay Buffer.

12 Prepare 50 µl of Reaction Mix for each reaction. Prepare an amount of Reaction Mix sufficient for all samples including ADP standards.

Component	Volume (µl)
ADP Assay Buffer	44
ADP Probe	2
ADP Converter	2
ADP Developer	2

13 Add 50 µl of ADP standards or samples per well to a clear 96-well plate.

14 Add 50 µl of Reaction Mix to each well.

15 Incubate for 30 min at room temperature.

16 Measure the absorbance at 750 nm using iMark™ Microplate Absorbance Reader.