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ADP Glo Max measuring ATP13A2 ATPase activity in microsomes

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ABSTRACT

Measuring ATP13A2 ATPase activity in microsomes using commercial luminescence-based ADP detection assay (ADP Glo Max; Promega)

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MATERIALS TEXT

Luminescence-based ADP detection assay (ADP Glo Max assay; Promega)

Reaction Buffer
50 mM MOPS-KOH pH 7.0
100 mM KCl
11 mM $MgCl_2$
1 mM DTT
0.02% DDM
0.001-10 mM spermine

1 Thaw microsomes on ice

2 Use 1 µg microsomes per reaction (1 µL of 5 mg/mL stock)

3 Dilute to 4 µL in Reaction Buffer at 4C

1h 40m

4 Equilibrate microsomes in reaction buffer for **01:30:00** **On ice** and for **00:10:00** at **37 °C**

5 Add 1 µL of 25 mM ATP (final concentration 5 mM ATP) to start reaction (total reaction volume 5 µL)

15m

6 Incubate for **00:15:00** at **37 °C** upon addition of ATP

5m

7 Terminate reaction by heating samples for **00:05:00** at **80 °C**

8 Mix with 5 µL of ADP-Glo Reagent for 60-80 min at **23 °C**

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9 Mix with 10 uL of ADP-Glo Max Detection Reagent for **01:00:00** 1h

10 Measure luminescence in 384 multi-well plate using a luminometer (NOVOstar; BMG Labtech)

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