ATP13A2 Luc-ATPase protocol (2016)

Reagents

4x reaction buffer (100 mL)

- 200 mM MOPS (4.184 g)
- 400 mM KCl (2.982 g)
- 44 mM $MgCl_2$ (0.894 g) [free Mg^{2+}: 6.1 mM]
- *4 mM DTT (add prior to use buffer 40 µL of 500 mM stock solution for 5 mL)*
- Adjust to pH 7.0 with KOH.
- (Per 96-well plate 1.2 mL 4x reaction buffer is used.)

ADP-Glo Reagent

ADP-Glo Max Detection Reagent

100 mM Ultra Pure ATP

10 mM ADP

Allow ADP-Glo Max Assay components to reach room temperature before use!

Assay

Reaction mix/well:

TOTAL: 25 µL reaction volume

- 1x reaction buffer (6.25 µL of 4x reaction buffer)
- 0.5 µg purified ATP13A2 OR 5 µg SHSY5Y-hATP13A2 OE microsomes
- for microsomes: add 2.5 µg DDM (1:2 DDM:protein ratio)
- compound
- *5 mM ATP (1.25 µL of 100 mM Ultra Pure ATP)*

Work flow:

1. pipet everything together in a white 96-well plate, except ATP

2. make 250 µL of 5 mM ADP (125 µL 10 mM + 125 µL 1x reaction buffer)
3. make 600 µL of 5 mM ATP (30 µL 100 mM + 570 µL 1x reaction buffer)
4. prepare the 5 mM series of ATP + ADP standards
5. transfer 25 µL into corresponding wells in duplo

Well number	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**
5 mM ADP (µL)	60	48	36	24	12	6	3	2.4	1.8	1.2	0.6	0
5 mM ATP (µL)	0	12	24	36	48	54	57	57.6	58.2	58.8	59.4	60

% ADP	100	80	60	40	20	10	5	4	3	2	1	0
% ATP	0	20	40	60	80	90	95	96	97	98	99	100

6. 1h incubation on ice

7. incubate for 5 min at 37°C

8. add ATP (5 mM final)
   Never exceed 5 mM, interferes with assay!

9. incubate for 20 min at 37°C

10. incubate for 10 min at RT

11. stop reaction with 25 µL ADP-Glo Reagent

12. incubate for 40 min at RT

13. add 50 µL ADP-Glo Max Detection Reagent and mix

14. incubate for 60 min at RT

15. read luminescence at FlexStation
    i. end point measurement
    ii. read mode: luminescence
    iii. integration: 500 ms
    iv. top read