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ATPase assay/ADP-glo kit

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We use this protocol and it's working

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Abstract

This protocol is successfully used to assess activity of P5B-ATPases with polyamines as substrate.

Materials

ADP-glo max assay (Promega, V7002)
DDM (Inalco, 1758-1350)

1 Prepare master mix on ice: 5 µg microsomes, 2.5 µg DDM (Inalco, 1758-1350), reaction buffer (50 mM MOPS-KOH, pH 7, 100 mM KCl, 11 mM $MgCl_2$ supplemented with 1 mM DTT and protease inhibitor (Sigmafast, Sigma, S8830-20TAB).

2 Incubate master mix for 30 minutes at room temperature in overhead shaker.

3 Pipet master mix (18.75 µl) into a white 96-well plate.

4 Prepare serial dilution of the substrate to be tested in 0.1 M MOPS-KOH, pH 7.

5 Pipet substrate dilution series (5 µl) into the 96-well plate.

6 Incubate for 5 minutes at room temperature then 5 minutes at 37 °C.

7 Add 5 mM pure ATP (1.25 µl) to initiate the reaction.

8 Incubate for 20 minutes at 37 °C followed by 10 minutes at room temperature.

9 Stop the reaction by addition of 25 µl ADP-glo reagent.

10 Incubate at room temperature for 40 minutes.

11 Add 50 µl/well of ADP-glo max reagent.

12 Incubate in the dark at room temperature for 60 minutes.

13 Measure luminescence (integration: 500 ms)