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ATP/NADH-enzyme coupled ATPase assay

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ABSTRACT

ATP/NADH-enzyme coupled ATPase assay to determine activation of purified ATPase protein via kinetic absorbance measurement.

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1 Purify the ATPase protein, flash freeze in liquid N2 and store at **-80 °C** until use.

2 Use a 384-well clear polystyrene microplate: add **25 µL** of the substrate to be tested, together with **40 µL** reaction mix, and add **10 µL** of **63 millimolar (mM)** ATP at **7.0** per well, with a final volume of **75 µL** per well.

2.1 Prepare serial dilutions of the substrate to be tested, in a final volume of **25 µL** per well.
If the substrate is dissolved in DMSO, keep the final DMSO concentration in **75 µL** reaction 0.2%.

2.2 Prepare **40 µL** of reagent mix per well containing: **50 millimolar (mM)** MOPS-KOH (**7.0**); **100 millimolar (mM)** KCl; **30 millimolar (mM)** MgCl2; 2.4 U/µL pyruvate kinase; 2.4 U/µL lactate dehydrogenase; **1.67 millimolar (mM)** PEP; and **0.6 millimolar (mM)** NADH, in the presence or absence of **600 ng** purified ATPase protein. Keep all compounds and the reagent mix at **4 °C** .

2.3 Add **10 µL** of **63 millimolar (mM)** ATP at **7.0** per well, and quickly proceed to the acquisition.

3 Mix the 384-well microplate for **00:00:15** prior kinetic measurement in an absorbance plate reader, set at **25 °C** . 15s

4 Measure absorbance at 340 nm, at **25 °C** for **00:30:00** to **01:00:00** . This results in at least 10 data points in the linear phase that can be plotted out over time to determine the OD 340 slope reduction. 1h 30m

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