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ATP/NADH-enzyme coupled ATPase assay

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We use this protocol and it's working

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Abstract

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

Troubleshooting

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