

NADH-coupled ATPase assay

Make the following stock solutions:

100 mM ATP (pH 7.0) = Dissolve 1.2 g ATP in 15 mL Milli-Q
Adjust pH to 7.0 with 0.1 N NaOH
Bring volume to 20 mL with Milli-Q
Make 1-mL aliquots and store at -80°C

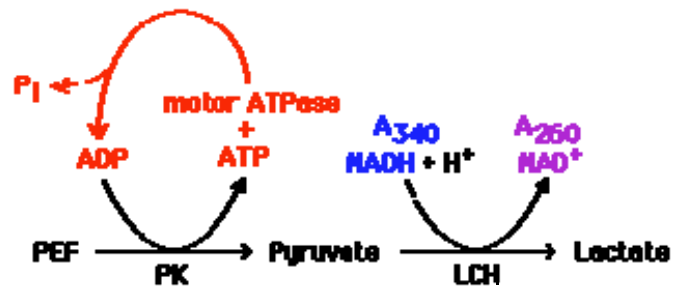
10 mM PEP = Dissolve 21 mg Phospho(enol)pyruvic acid in 10 mL Milli-Q
Make 1-mL aliquots and store at -80°C

10 mM NADH (pH 8.0) = Dissolve 71 mg β -Nicotinamide adenine dinucleotide in 10 mL 25 mM Na-HEPES, pH 8
Make 1-mL aliquots and store at -80°C

2x ATPase buffer = 50 mM Na-HEPES (pH 8)
10% Glycerol
100 mM NaOAc (pH 7.5)
300 μ M NaCl
0.02% NP-40

Set up two master mixes on ice:

<u>Tube A</u>	<u>Tube B</u>
Helicase	10 mM ATP
DNA (optional)	10 mM MgCl ₂
Buffer (1x final conc.)	1 mM PEP
Milli-Q water to 60 μ L	0.8 mM NADH
	10 U/mL PK
	16 U/mL LDH
	Buffer (1x final conc.)
	Milli-Q water to 60 μ L



All of the concentrations are 2x because they're diluted by half when the master mixes are combined.

1. Mix contents of Tubes A and B to start the reaction.
2. Load 100 μ L into 96-well plate.
3. Monitor absorbance at 340 nm at the desired temperature.

NADH and PEP are consumed in a 1:1 ratio during the reaction to produce a change in A₃₄₀. By doubling their concentrations, the linear range of the reaction can be doubled (useful for fast reactions).

If a reaction ends (*i.e.*, runs out of NADH) in < 10 min, it may be approaching the maximal rate of the PK and LDH enzymes, and therefore, they may become rate limiting. In this case, the reaction needs to be adjusted to increase the time domain. If the reaction cannot be slowed, the PK and LDH enzyme concentrations can be increased to compensate. If NADH and PEP are doubled, then reactions ending in 20 min may be approaching the rate limit of PK and LDH, and the same adjustments need to be made.