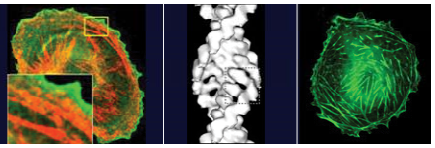

NADH Enzyme-Linked Assay

$$\text{ADP} + \text{Phosph(enol)pyruvate} \xrightarrow[\text{Pyruvate Kinase}]{} \text{ATP} + \text{Pyruvate} \xrightarrow[\text{Lactate Dehydrogenase}]{\text{NADH} \circlearrowright \text{NAD}} \text{Lactate}$$

Stock Solutions

4000 U/ml	Lactic Dehydrogenase (Sigma L-1254), store in freezer in 50% glycerol
10,000 U/ml	Pyruvate Kinase (Sigma P-9136), store in freezer in 50% glycerol
100 mM	Phospho(enol)pyruvate (Sigma, P-7127), store in freezer in water
1 mg	NADH, reduced form (Sigma, N0786), store at room temperature in the dark

5x Cocktail Solution

Add 1.2 ml assay buffer (usually KMg50 or KMg10) to 1 mg NADH, and add:

32 μl	Lactic Dehydrogenase
64 μl	Pyruvate Kinase
32 μl	Phospho(enol)pyruvate

This will result in the following concentrations:

1000 μM	NADH
100 U/ml	Lactic Dehydrogenase
500 U/ml	Pyruvate Kinase
2.5 mM	Phospho(enol)pyruvate

Assay

1. When using the stopped flow, one syringe should contain a 2x mixture of the Cocktail Solution and the appropriate 2x MgATP concentration (usually 4 mM).

Example of 2x Cocktail (2.88 ml):

1200 μl	5x Cocktail from above
115 μl	0.1 M ATP
11.5 μl	1 M $MgCl_2$
1550 μl	Assay Buffer

2. Detect absorbance change at 340 nm, or detect fluorescence emission (Ex. 340 nm) with 400 nm long-pass filter.

3. Use extinction coefficient for NADH (ε_{340} = 6220 $M^{-1}cm^{-1}$) to determine rate of ADP production (NADH loss). Remember that the path length of the stopped-flow cuvette is 0.2 cm.

4. When using fluorescence detection, a standard curve with known ADP concentrations must be obtained.