# Lactate Assay

***Biochemistry:***

Lactate is oxidized to pyruvate by the lactate dehydrogenase (LDH) reaction. The hydrazine destroys the pyruvate, allowing the reaction to run to the complete oxidation of all lactate molecules. To ensure this, $NAD^+$ is provided in excess. The concentration of lactate in the sample is proportional to the increase in absorbance as $NAD^+$ is reduced to NADH.

***Sample Preparation:***


The blood/tissue sample needs to be deproteinized to prevent the slow release of lactate from erythrocytes. The deproteinization is best done by a small (1:2 or 1:3) dilution in 6% PCA. Alternatively, blood can be treated with an anti-glycolytic agent (sodium fluoride), left to clot, and centrifuged for subsequent collection of serum. The anti-glycolytic agent could also be used with heparin or EDTA to block clotting, with centrifugation and subsequent collection of plasma.

**Table 1: Assay cocktail ingredients (designed for glycerol ingestion studies).**

| **Compound** | **Final []** | **Amount of Compound for given cocktail volume** | | | | |
|---|---|---|---|---|---|---|
| | | ***25 mL*** | ***100 mL*** | ***150 mL*** | ***200 mL*** | ***250 mL*** |
| Water | | 16 | 64 | 96 | 128 | 160 |
| Glycine | 320 mmol/L | 8 mL | 32 mL | 48 mL | 64 mL | 80 mL |
| Hydrazine | 320 mmol/L | 0.4 mL | 1.6 mL | 2.4 mL | 3.2 mL | 4 mL |
| $NAD^+$ | 2.4 mmol/L | 0.00955 g | 0.0382 g | 0.0573 g | 0.0764 g | 0.0955 g |
| ***Enzymes*** | | | | | | |
| LDH* | 2 U/mL | 10 µL | 40 µL | 60 µL | 80 µL | 100 µL |

* LDH stock = 5,435 U/mL

Use distilled water samples for the blanks. Read absorbance at 340 nm, and first zero to the blanks. Calculate lactate from the Beer-Lambert Law, using 6.22 as the millimolar extinction coefficient for NADH. Resting blood samples should approximate 1 mmol/L.

**Table 2: Stock Sample Locations**

| **Fridge** | **Freezer** | **Bench** |
|---|---|---|
| Glycine solution (1 M), LDH | $NAD^+$ | Hydrazine (from store room cabinet) |