

## II. SOLUTIONS

### A. Stock Solutions

The following stock solutions are available:

**Buffer Solution** (to control the pH of the reaction mixture)

- 0.024 M pyrophosphate pH 8.8 buffer (made from  $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$  and  $\text{H}_3\text{PO}_4$ )

**ADH Solution** (the enzyme solution)

- X  $\mu\text{g}/\text{mL}$  ADH in a 0.01 M pH 7.5 potassium phosphate buffer (you will be provided the value of X which is typically 1 to 15).
- Dilute solutions of ADH rapidly lose activity upon standing and are quite unstable at pH values below 6.0 or above 8.5. The deactivation rate is reduced by storing the solutions at 0-5 C when not in use and by placing the enzyme solution container in an ice bath during lab work.

**NAD<sup>+</sup> Solution** (coenzyme)

- 0.017 g/mL (2.5 mM) in  $\text{H}_2\text{O}$
- This solution is stored in the refrigerator when not in use and in an ice bath during lab work.

**Ethanol Solutions**

- 0.2500 M (14.58 mL of 100% ethanol (MW, 46.07; density, 0.790) / L)
- 0.1000 M
- 0.01250 M
- 0.003125 M
- These stock solutions of ethanol are prepared in 0.024 M, pH 8.8 pyrophosphate buffer.

### B. Preparation of Standard Ethanol Solutions

Rinse all glassware thoroughly as soap can denature the enzyme. Prepare the following standard ethanol solutions from the 0.01250 M (12.50 mM) ethanol stock solution in 100-mL volumetric flasks (note standard solution concentrations are given in millimolar):

Final concentration of EtOH standard solution mM	Volume of 12.50 mM EtOH stock solution from buret mL	Final volume (dilute to mark with pH 8.8 buffer) mL
0.125	1	100.0
0.250	2	100.0
0.625	5	100.0

All these solutions should be diluted to volume with the pH 8.8, 0.024 M, pyrophosphate buffer. Burets have been set up to dispense the 12.5 mM ethanol solution. Record the beginning and ending buret readings (to  $\pm 0.01$  mL) and calculate the exact volumes dispensed and exact concentrations later to be used for analysis. It is very critical that all glassware used in the preparation of the solutions is very clean and that you minimize contamination of the solutions with particulate matter (e.g., dust, hairs, skin).

### C. Preparation of Beer Sample

The real beer must be diluted by a factor of 5000 and non-alcoholic beer by a factor of 500 to ensure that the ethanol concentration in the standard solution is in the region where  $v_0$  is proportional to  $[S]_0$  (i.e., equation 4 is reasonably valid.)

It is difficult to pipet an exact volume of beer since a head forms and obscures the meniscus. Hence the bubbles ( $\text{CO}_2$ ) must be removed. Place a few milliliters of beer in a test tube, cover with a 1" square piece of Parafilm, shake vigorously, and uncover to release the gas pressure. Repeat a few times until foaming ceases. Alternatively, use a sonificating bath if available.

With a pipet, transfer 1.00 mL of flat beer (or 10.00 mL of non-alcoholic beer) into a 100-mL volumetric flask and dilute to volume with deionized water. Transfer 2.00 mL of this solution (cleaned pipet) to another 100-mL volumetric flask and dilute to volume with pH 8.8, 0.024 M, pyrophosphate buffer (not water).

*Solution Preparation for blood sample and standards for blood analysis.*

*Unknown.* Rinse glassware thoroughly as soap can denature the enzyme.

Obtain a centrifuge tube from the TA and ask the TA to dispense 1 mL of blood into the centrifuge tube. Add 5.0 mL of 6% TCA. With a stirring rod, break up all blood clumps as completely as possible. Centrifuge for 3 min.

Transfer 2.0 mL of the clear supernatant to a 25-mL volumetric flask and add 1 drop of magenta-colored thymol blue (non-ethanol solution). Add a drop of 0.5 M NaOH, shake the contents, wait 10-15 s for a color change. Continue to add drops of 0.5 M NaOH, shake and wait until the solution turns from red to yellow (about 13-30 drops total-record the number of drops in your laboratory notebook). Now use the more diluted 0.1 M NaOH and add dropwise with a Pasteur pipet (shaking after each drop) until the first faint green-blue color appears. The pH is now near the pH used for the standard assay procedure. ***Dilute with pH 8.8 buffer to the 25-mL mark.***

*Standards.* In 10-mL volumetric flasks, prepare 0.030 M and 0.060 M alcohol standard solutions from the stock 0.25 M ethanol solution (1.2 and 2.4 mL of 0.25 M EtOH with the electronic pipet and ***dilute to 10 mL with the pH 8.8 buffer***). Record actual values and calculate the alcohol concentrations later.

To make the ethanol standards appropriate for the analysis of blood (i.e., similar in solution composition and pH to the blood samples), mix 1 mL of each EtOH standard with 5.0 mL of 6% TCA. Transfer 2.0 mL of this mixture to a 25-mL volumetric flask, add thymol blue, adjust the pH, and **bring up to volume with buffer**. **Note that after sample treatment, the concentrations for the two standards are 0.4 mM and 0.8 mM in ethanol in the standard solutions.**

*Analysis*

Run duplicate assays of the treated blood sample and two treated standards by adding 250  $\mu\text{L}$  of NAD to 2.00 mL of sample or standard and injecting 250  $\mu\text{L}$  of ADH (runs 17-22).

Return the sample cells with stir bars to a TA.