Alcohol Dehydrogenase (ADH) Assay Kit

(Cat/No.:BC122 Size:50T/48S)

1. Principle of Measurement

ADH catalyzes the reduction of ubiquinone 1 to ubiquinol 1 with alcohol as a reducing agent. This will change the absorbance value at 340 nm. Based on such change, the activity can be calculated.

2. Compositions and Preparation (The kit is valid for 3 months)

Reagent I: 1 Bottle×20ml. Preserved at 4°C.

Preparation of Reagent I Standard Solution: Dilute reagent I with the same amount (Volume) of double distilled water (DDW) prior to use.

Reagent II: 1 Bottle×3ml. Preserved at 4°C.

Reagent III: 4 Bottles of Powder. Preserved at -20°C.

Preparation of Reagent III Solution: Dissolve one bottle of powder with 10 ml DDW and blend the mixture till fully dissolved right before use. Preserved -20°C.

3. Procedures

Compositions (ml)	Blank	Sample
Reagent I Standard Solution	0.65	0.65
Reagent II	0.05	0.05
Reagent III Solution	0.75	0.75
Mix thoroughly and warm the mixture in a water bath at 37°C for 10 min.		
Sample (Serum or Plasma)		0.05
DDW	0.05	

Record the time right after the addition of samples. Mix the solution thoroughly and 15 seconds after the addition, record the absorbance A_1 of each tube at 340 nm with 0.5 cm path length.

Warm the mixture in a water bath at 37°C and 20 minutes and 15 seconds after timing, record the absorbance A_2 of each tube, $\triangle A=A2-A1$.

4. Detailed Procedures

I. Preparation of Working Fluid: Mix reagent I standard solution, reagent II and reagent III solution with the ratio of 0.65:0.05:0.75 (v/v) with the exact amount needed prior to use.

II. Zero the cuvettes with DDW using spectrophotometer, the path length of the cuvette is 0.5 cm.

III. Transfer 50 µl serum/plasma sample and DDW to the corresponding test tube. Then transfer 1.45 ml working fluid prepared to each test tube and start timing. Mix the solution in the test

tube.

IV. Transfer the mixture into the corresponding cuvette and record the absorbance value A_1 15 seconds after timing.

V. Place the cuvettes into the corresponding thermostatted compartment with the surrounding temperature equals to 37°C. Were the compartment unavailable, transfer the mixture back to the test tube and warm the solution in a water bath at 37°C.

VI. 20 minutes 15 seconds after timing, record the absorbance value A_2. Before the measurement, transfer the mixture back to the cuvette if necessary.

VII. Calculate the absolute A values, $\Delta A=A_2-A_1$.

5. Calculation Formula and Example

I. Definition: One activity unit is defined as 1 nmol product generated catalyzed by enzymes within 1ml serum/plasma sample.

II. Calculation Formula

$$\underset{U/ml}{Enzyme\ Activiy} = \frac{\Delta A_{sample} - \Delta A_{Blank}}{MAC} \div \frac{Path\ Length}{0.5cm} \times \frac{V_{total}(1.5ml)}{V_{sample}(0.05ml)} \div \frac{t}{20\ min} \times 1000$$

Note: MAC represents the milli-molar attenuation coefficient and is 6.22 $mM^{-1}\cdot cm^{-1}$.

III. 0.05ml human serum was extracted and measured. The A_1values were 0.165 and 0.137 respectively and the A_2 values were 0.166 and 0.163 respectively.

$$\underset{U/ml}{Enzyme\ Activiy} = \frac{\Delta A_{sample} - \Delta A_{Blank}}{MAC} \div \frac{Path\ Length}{0.5cm} \times \frac{V_{total}(1.5ml)}{V_{sample}(0.05ml)} \div \frac{t}{20\ min} \times 1000$$

$$= \frac{(0.163 - 0.137) - (0.166 - 0.165)}{6.22} \div 0.5 \times \frac{1.5}{0.05} \div 20 \times 1000 = 12.06 U/ml$$

6. Significance

95% ADH exists in centrilobular of liver and 80-90% can be found within hepatic cytoplasm. A small part of the ADH can be found in microsomes.