## Module 3 : Biochemistry and Life

In this module, there is an animation showing the principle of enzyme-linked immunosorbent assay (ELISA) for the measurement of plasma insulin. Also, case studies on green tea antioxidant and toxicity of pesticides are employed to demonstrate some daily applications related to biochemistry.

After going through this module, you will know more about the investigation approaches of this kind of applications.

## **3a. Introduction**

Biochemistry is closely related to our daily life. Our diet can affect many physiological condition of our body through different biochemical reactions, some may be good to our health, and however, some may bring toxic effects.

In this module, we are going to learn more about the toxicity of pesticides that always are present on vegetables; effect of green tea antioxidant on lipid oxidation in red blood cell membrane. Also, ELISA (Enzyme-linked Immunosorbent Assay) will be employed for the measurement of plasma insulin concentration.

## **3b. Relative toxicology of pesticides**

# Relative toxicology of pesticides: Toxicology study of cholinesterase by spectrophotometer

Pesticides on vegetables always cause toxic effect on human who have taken it. The effect of pesticides is inhibiting the activity of cholinesterase in serum, which convert acetylcholine to choline.

By adding 5,5'-dithio-bis-2-nitrobenzoate (DNTB) to choline, thionitrobenzoic acid, which is a yellow chromogen forms. It absorbs light at 412 nm. The effect of pesticides can be inversely determined by reading of spectrophotometer at 412 nm.



#### Video : Toxicology study of cholinesterase (see website)

### **3c. Tea antioxidants**

Oxidation of membrane lipid, protein, and nucleic acids can resulted in loss of membrane integrity and function, inactivation of enzymes, modification of lipoproteins, and chemical alteration of DNA, cause aging or even diseases like cardiovascular and cancer disease. Therefore, foods with antioxidant are highly recommended to be included in our meal.

Green teas are known to contain natural antioxidant. In this module, we are going to investigate the effect of the antioxidant in green teas, also, its effect will be compared with black teas.

# Video : Effect of tea antioxidants on lipid oxidation in red blood cell membrane (see website)

### 3d. Measurement of plasma insulin by ELISA

Enzyme-linked Immunosorbent Assay (ELISA), based on two monoclonal antibodies. Simultaneous incubation of sample and enzyme-labelled antibody in a microplate well coated with a specific anti-insulin antibody forms a complex. The bound conjugate is detected by reaction with the substrate. 3,3',5,5'-tetramethylbenzidine(TMB) is to be added in the final step as a substrate for HRP. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at 450nm.

The inclusion of calibrators of known insulin concentration in the assay allows a calibration curve to be constructed from which the level of insulin in samples can be determined.

#### Animation: 3d Measurement of plasma insulin by ELISA (see website)