***[BCH 322]***

***Method of Enzyme Assay***

***1-*** ***Discontinuous assay (colorimetric assay)***

***Method***

**Type of cuvette used --------------------**

Prepare each tube as follows:

|  |  |  |
| --- | --- | --- |
|  | **BLANK** | **SAMPLE** |
| **ALT Reagent** | 0.5 ml | 0.5 ml |
| Pre-warm at 37 °C for 5 minutes and add: | | |
| **Distilled Water** | 0.1 ml | - |
| **Serum Sample** | - | 0.1 ml |
| Mix, and incubate at 37 °C for exactly 30 minutes, and add: | | |
| **Color Reagent**  **(DNPH)** | 0.5 ml | 0.5 ml |
| Mix, and return at 37 °C for exactly 10 minutes, then add: | | |
| **Color Developer**  **(NaOH)** | 5.0 ml | 5.0 ml |
| Mix, and return to 37 °C for exactly 5 minutes. Read absorbance of all tubes at 546nm against blank. | | |

***Result***

Absorbance at 546 nm = ………

ALT (SGPT) activity of serum sample (from graph)= …………U/L

***2-*** ***Continuous assay (UV/ kinetics)***

***Method***

**Type of cuvette used --------------------**

Prepare the following:

|  |  |
| --- | --- |
| **ALT Reagent** | 3 ml |
| Pre-warm at 37°C for 3 minutes and add | |
| **Serum Sample** | 0.2 ml |
| Mix and incubated at 37 °C for 1 minute, then read absorbance (at 340 nm against distilled water) every minute for 3 minutes) and determine ∆A/min | |

**Choose the following on the spectrophotometer:**

**2) Applications 🡪 2) Simple Kinetics 🡪 wave length (340 nm) 🡪 1) Seconds 🡪 Duration (180 sec = 3 min) 🡪 Intervals (60 sec= 1 min) 🡪 Print Data Table (off) 🡪 Press start (2 times)**

***Result***

|  |  |  |  |
| --- | --- | --- | --- |
| ***Time*** | ***Absorbance 340nm*** | | ***ΔA/min=((A1-A2)+(A2-A3))/2*** |
| ***1 min*** | ***A1*** |  |  |
| ***2 min*** | ***A2*** |  |
| ***3 min*** | ***A3*** |  |

***Calculation***

ALT Activity ( U/L) = ΔA/min x 1768

ALT Activity = ……………..U/L