Assessed practical BI301:

Pipette 3ml of Phosphate-malate buffer (0.05M) into a silica (quartz) spectrophotometer cuvette (glass or plastic is uv opaque). Place the cuvette in the spectrophotometer (wavelength setting = 250nm) and zero the instrument. Pipette 10 µl of enzyme solution into the cuvette and mix well. Record the changes in extinction every 10 sec over a 60-180 sec period or use the recorder. Calculate the average extinction increment per min arithmetically or from the slope of the graph of E $\underline{vs}$ time. Convert your rate (extinction/min) to µM/min (extinction coefficient 1450 $1.mol^{-1}$ cm^{-1}). Repeat the determination three times and calculate an average rate. Calculate the specific activity value (the concentration of fumarase in your stock will be given to you on the day).

Example:

1. Determine the initial rates (the difference in OD/min) using the tangent on the chart recorder printout:
enzyme (0.010 ml): 0.02 / 15 sec = <u>0.08/min</u>

2. Calculate the amount of µmoles / litre /min:
E = e x c x d
0.08 / min = 1450 mol^{-1} cm^{-1} l x c x 1 cm
c = 0.08 /1450 mol/l min = <u>55.2 µmol/l min</u>

3. Production of nmol / min:
You produce 55.2 µmol/l min, how many moles are in 3 ml?
55.2 µmol/l min x 0.003 l = <u>165.5 nmol / min</u>.

4. Specific activity (µmol/ min mg):
Enzyme concentration is 180 µg / ml and you used 0.01 ml, therefore you have got
180 µg/ ml x 0.01 ml = 1.8 µg of enzyme,
which give you a change of <u>165.5 nmol / min</u>.

The specific activity therefore is:
165.5 nmol / min / 1.8 µg = <u>0.092 mol / min g (= 92 µmol / min mg)</u>.

COSHH Regulations 1988. This instruction sheet has been subjected to a formal risk assessment. **<u>Safety specs and gloves must be worn at all times</u>**.

Signed: Date: