## **SOD ASSAY**

## Based on the inhibition of the oxidation of epinephrine

Summary of equations for data analysis % I = % INHIBITION  $(V_o - V_{SOD}) * 100 / V_o = 1 - (V_{SOD} / V_o)$ 

For every assay find SOD<sub>50</sub> and -->

- SAMPLE SPREADSHEET
- 1. Print spreadsheet on one page with SOD<sub>50</sub> calculated.
- 2. Print both the Inhibition plot and the double reciprocal plot with the theoretical lines indicated.

HYPERBOLIC PLOT **INVERSE PLOT** DATA FOR THE INVERSE PLOT DATA FOR PERCENT INHIBITION PLOT FOR STANDARD SOD (double reciprocal plot)  $(V_{SOD}/V_{o)} \times 100$ INVERSE INVERSE % OF V<sub>o</sub> % OF V<sub>o</sub> SOD = % I % I SOD % I % I 10<sup>-9</sup> M calc calc'd for calc'd for (or nM) theor. line theor. line X Yobs Y<sub>calc</sub> Χ Yobs Ycalc V<sub>o</sub>\* 0.0000 0.00 0.00 0.010700 100.0 100.0 82.5  $\mathsf{V}_\mathsf{sod}$ 0.1950 0.008810 82.3 17.7 17.5 5.13 0.0566 0.0571 0.3900 0.007560 70.7 69.6 29.3 30.4 2.56 0.0341 0.0329 0.7780 0.005570 52.1 51.9 47.9 48.1 1.29 0.0209 0.0208 1.1700 0.004200 39.3 40.2 60.7 59.8 0.855 0.0167 0.0165 1.5500 0.003110 29.1 32.1 70.9 67.9 0.645 0.0141 0.0147 1.9300 0.002630 24.6 26.1 75.4 73.9 0.518 0.0133 0.0135 " 16.1 15.3 2.9800 0.001720 83.9 84.7 0.336 0.0119 0.0118 11 5.85 94.1 4.7600 0.000870 8.13 91.9 0.210 0.0109 0.0106 0.00 0.0086 Calculated for demonstration only Skip in routine analyses for inverse plot [SOD] b Show all Vo values for runs  $Y_{calc} =$ m  $Y_{calc} = \{ m + b*[SOD] \}$ [SOD] in spreadsheet; use average for calculations of %I  $Y_{calc} =$ for inhibition plot = mx + bAt low SOD,  $b*[SOD] \ll m$ , so Y = (1/m)\*SODwhere x = 1/SODAt high SOD,  $b^*[SOD] >> m$ , so Y = (1/b)Summary Output Inverse Plot of Yobs vs X  $SOD_{50} = 50 * m / (1 - 50* b)$ Regression Statistics Multiple R 0.9993 use m and b from the slope and R Square 0.9987 Find these Adj R<sup>2</sup> % errors y-intercept of the inverse plot 0.9984 For the data above: Std Error 0.000623 Obsns SOD<sub>50</sub> = 0.832 x 10<sup>-9</sup> M Coefficients Std Error % Int = b 0.00864 0.00030 3.5 50 \* (0.00945 x 10<sup>-9</sup> M) 0.00945 0.00014 1.5 X var 1 = m{ 1 - 50 \* (0.00864)

1.09 x 10<sup>-9</sup> M

m/b =

than 90%, use this definition of a unit of SOD activity

 $SOD_{50}' =$ 

If the maximum percent inhibiton is less

0.00945 x 10<sup>-9</sup> M

This is higher than the 90 - 100 % expected

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Max % Inhibition = 1/b =