

Report (Journal Format with Text Typed)

explain the principles behind this method

Your report should include:

Intro

1. Method used to prepare ammonium sulfate suspension. *↑ what is about?*
2. Batch Affi-Gel Blue method used to purify LDH and enzyme assay procedure. Explain also why Affi-Gel Blue can be used to bind and purify LDH. What step of the purification (on Day 2) allows LDH to be eluted from the affinity media and why? Be specific.
3. General description of protein analysis procedure.
4. General description of enzyme kinetic procedures.

Items 1-4 should be in the "Introduction" section of the report.

Results

5. Standard curve for protein determination Bradford Assay. Protein concentrations (mg/mL) of B-1 solution and Affi-Gel Blue purified LDH (B-11 solution). See the following page for sample calculation.
6. Determination of LDH activity ("total activity", in LDH units/mL) of B-1 solution and Affi-Gel Blue purified LDH (B-11 solution). See the following page for sample calculation.
7. Determination of specific activity of B-1 solution and Affi-Gel Blue purified LDH (B-11 solution). See the following page for sample calculation.
8. Determination of purification factor.

$$\text{purification factor} = \frac{\text{specific activity of Affi-Gel Blue purified LDH (B-11 solution)}}{\text{specific activity of B-1 solution}}$$

9. Determination of % LDH recovery from Affi-Gel Blue purification procedure.

$$\% \text{ LDH recovery} = 100 \times \frac{\text{UNITS of LDH activity recovered from Affi-Gel Blue}}{\text{UNITS of LDH activity added to Affi-Gel Blue}}$$

NOTE: Assume 2.0 mL of purified LDH solution recovered from Affi-Gel Blue.

10. Determination of total units of LDH activity in your original ammonium sulfate suspension.
11. Table of initial rate data: $[S_0]$, v_0 (\pm inhibitor), $1/[S_0]$, $1/v_0$ (\pm inhibitor), $v_0/[S_0]$ (\pm inhibitor, if use Eadie-Hofstee plot) and $[I]$.
12. Graph of v_0 vs. $[S_0]$ data with and without inhibitor (on one graph). Estimate K_m and V_{max} . V_{max} units = $\Delta A_{340}/\text{min}$.
13. Lineweaver-Burk or Eadie-Hofstee plot of "linear transformed" v_0 vs. $[S_0]$ data with and without inhibitor to determine K_m and V_{max} . V_{max} units = $\Delta A_{340}/\text{min}$. Calculate K_i only if competitive or non-competitive inhibitor. Be sure to include calculations of V_{max} 's, K_m 's and K_i (if applicable) in your appendix.

Discussion

14. Comparison of V_{max} 's and K_m 's obtained from v_0 vs. $[S_0]$ plot and Lineweaver-Burk or Eadie-Hofstee plot. Provide a discussion of the differences and why they might be this way.
15. Report the type of inhibition observed for your unknown inhibitor and discuss with respect to the kinetic model most consistent with your results. Remember to report your Unknown Number.
16. Discussion of results with respect to what you might have expected. Discuss three difficult experimental procedures that might produce errors.

Items 14-16 should be in the "Discussion" section of your report.