Biomolecules of Life (Laboratory)

PROTEINASE K CRYSTALS WORKSHEET

Scoring:
0 Clear drop
1 Non-protein particles
2 Some precipitates
3 Phase separation
4 Microcrystals
5 Salt crystals
6 Single crystals
7 Defective crystals
B Birefringence

Your Names: Emily Zhang, Cindy Qu. Date: June 18th 2025

Q1. In each box below, corresponding to the well containing the drop, record the score that best classifies the contents of the drop. If there are multiple crystals, use ";" to separate each score. Describe the crystal morphology (shape, appearance) with your score. Note that each drop can have multiple numbers and letters recorded. **(12 pts)**

A1 3, 7B (Irregular pyramid)	A2 0	A3 0	A4 3, 6B (Irregular pyramid, smooth)	A5 3, 6B (Irregular pentagonal prism, smooth)	A6 2, 7B (Irregular pyramidal, smooth)
B1 3, 6B (Irregular hexagonal, smooth)	B2 2, 6B (Irregular pyramid, smooth)	B3 0	B4 0	B5 2	B6 2
C1 0	C2 2, 6B; 2, 6 (Irregular pyramid smooth; irregular pyramid smooth)	C3 1	C4 3, 7 (Irregular hexagonal)	C5 2	C6 1
D1 0	D2 0	D3 0	D4 0	D5 0	D6 3, 6B (Irregular rectangular prism)

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Your Names: Emily Zhang, Cindy Qu Date: 2025-06-23

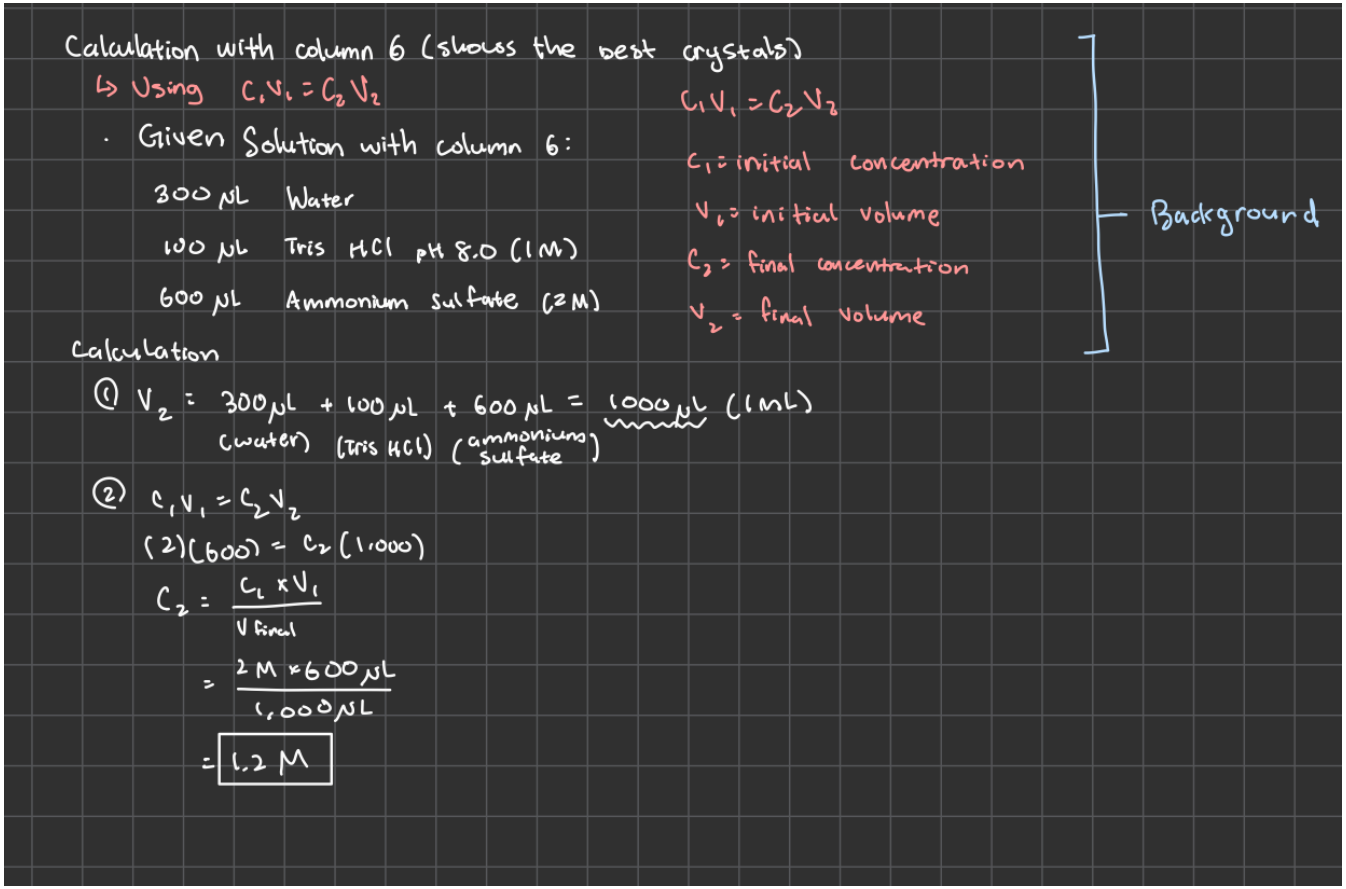
Q2. . In each box below, corresponding to the well containing the drop, record the score that best classifies the contents of the drop. If there are multiple crystals, use ";" to separate each score. Describe the crystal morphology (shape, appearance) with your score. Note that each drop can have multiple numbers and letters recorded. **(12 pts)**

A1 3, 7B cracked, tetrahedral	A2 0	A3 0	A4 6B tetrahedral, smooth	A5 6B tetrahedral, slight cracks	A6 7B two crystals merged, cracked
B1 6B trapezoidal	B2 6B tetrahedral, smooth	B3 0	B4 0	B5 2	B6 1
C1 0	C2 6B; 6 both octahedral	C3 1	C4 3, 7 mostly tetrahedral but very cracked	C5 2	C6 3 no crystals
D1 0	D2 0	D3 0	D4 1	D5 0	D6 6B tetrahedral, some dents (not cracks)

Q3. Based on your data, what is the optimal concentration of ammonium sulfate in the reservoir for future crystallization trials? Briefly show how you obtained your answer. (Hint: you should use the C1V1 = C2V2 equation). **(5 pts)**

Based on data, the optimal concentration of ammonium sulfate is 1.2 M because of our experimental results showing that column 6 shows the best crystals. As such, the concentration of the solution for column 6 was used to calculate the optimal concentration of ammonium sulfate.

The answer was obtained by first calculating the volume of the final solution (V2). Then, we plugged in given values for C1, V1 (from column 6) and the calculated V2 into the C1V1=C2V2 equation to solve for C2. The answer calculated for the optimal concentration of ammonium sulfate is then found to be 1.2 M (work shown below)



Q4. What is the optimal ratio of Proteinase K to reservoir solution that supports crystal growth? **(2 pts)**

The optimal ratio of Proteinase K to reservoir solution that supports crystal growth is 2.5:1.5 (2.5 microliters Proteinase K: 1.5 microliters reservoir solution). This is based on Row A, in which the formulation of the solution involved 550 microliters water + 100 microliters Tris HCl at pH 8.0 (1M) + 350 microliters ammonium sulfate (2M). This ratio from Row A was chosen to be optimal because it produced the highest crystal yield. This ratio is likely to be because the final ammonium sulfate concentration, which was 0.7 M after dilution, and buffer conditions created ideal supersaturation. Furthermore, the 2.5:1.5 ratio supported proper protein-precipitant equilibrium for nucleation, which is displayed by Row A outperforming other ratios in crystal quantity/quality.

Q5. How did the scoring change over the three occasions that you checked your drops? Did any specific drops exhibit a slower crystal growth compared to the other drops? **(2 pts)**

Scores remained consistent across observations, indicating stable crystallization conditions. However, crystal surfaces became smoother over time, likely due to oil droplet dissolution from phase separation. No drops exhibited significantly slower growth, suggesting uniform nucleation rates. (Slower-growing crystals might indicate suboptimal precipitant or protein concentrations, which we did not have)

Q6. Why is it important to set up control drops (e.g. water + precipitant only)? **(2 pts)**

Control drops are important to set up because they help differentiate crystals from precipitate (ex: salt vs protein). In addition, they can also help set the baseline in order to identify false positives such as phase separation and debris. The control drops also help confirm that crystallization is driven by the protein, and not other contaminating components or buffer components. As such, control drops are crucial in order to help identify the experimental results