

Optimization of Proteinase K
M230D, January 2024
Crystallization Core, Boyer Hall Room 106

RESERVOIR COMPOSITION: Set up a 24-well plate. Prepare the reservoir first.

| | COLUMN | 1 | 2 | 3 | 4 | 5 | 6 |
|----------|-----------------------|----------|----------|----------|----------|----------|----------|
| ROW | Solution | vol (uL) | vol (uL) | vol (uL) | vol (uL) | vol (uL) | vol (uL) |
| A Apo | Water | 450 | 450 | 400 | 400 | 350 | 350 |
| | Tris HCl pH 8.0 (1M) | 100 | 100 | 100 | 100 | 100 | 100 |
| | Ammonium Sulfate (2M) | 450 | 450 | 500 | 500 | 550 | 550 |
| B Apo | Water | 450 | 450 | 400 | 400 | 350 | 350 |
| | Tris HCl pH 8.0 (1M) | 100 | 100 | 100 | 100 | 100 | 100 |
| | Ammonium Sulfate (2M) | 450 | 450 | 500 | 500 | 550 | 550 |
| C Apo | Water | 450 | 450 | 400 | 400 | 350 | 350 |
| | Tris HCl pH 8.0 (1M) | 100 | 100 | 100 | 100 | 100 | 100 |
| | Ammonium Sulfate (2M) | 450 | 450 | 500 | 500 | 550 | 550 |
| D Apo | Water | 450 | 450 | 400 | 400 | 350 | 350 |
| | Tris HCl pH 8.0 (1M) | 100 | 100 | 100 | 100 | 100 | 100 |
| | Ammonium Sulfate (2M) | 450 | 450 | 500 | 500 | 550 | 550 |

Note: Using a tip, make a tiny cut on the grease of each reservoir well.

PREPARING PROTEINASE K: Obtain 100 uL of 30mg/mL proteinase K in water. Label your tube with your initials and centrifuge at 10,000 RPM for five minutes. After centrifugation, take 4 small Eppendorf and add 20uL of the supernatant in each of them.

IMPORTANT! DO NOT ASPIRATE FROM THE BOTTOM OF THE SAMPLE TUBE when transferring to the new tube. Avoid extracting centrifuged seed crystals from the original sample tube. This is crucial for obtaining large crystals.

GENERATE 24 HANGING-DROPS: Aspirate from the protein tube using the same pipette tip for each row of drops. Then aspirate from the reservoir solutions for each drop, changing pipette tips every time. **ASPIRATE FROM JUST BELOW THE LIQUID LEVEL** of your protein preparation.

DROP PLACEMENT: You will place the drops **ONE ROW** at a time. On each of the six cover slips, dispense the protein in the center. Do not exceed the first stop on the pipette. Quickly, but carefully change tips. From each of the reservoirs in your row, introduce the reservoir solution to the drops containing protein. Exchange tips between transfers, and place the cover slip carefully over the pre-greased reservoir after the six drops have been made.

CONCENTRATION/RATIO BY ROW: [Sample:reservoir]

Row A and B: 3uL of protein, and 1uL of reservoir. [3:1]

Rows C: 2.66uL of protein, and 1.33uL of reservoir. [2:1]

Row D: 2uL of protein, and 2uL of reservoir. [1:1]