



# Purification Planning Check Sheet



MSUM Biochemistry

**PURPOSE OF THIS DOCUMENT:** The Purification planning check sheet is intended to help the chromatographer plan their way through a purification step. It is not a laboratory book (although you should add this into your lab book) nor does it replace an understanding of the theory and concepts of biochemical separations. This sheet will greatly aid in an efficient preparation and execution of a purification step. Good luck and be well resolved!

## CHROMATOGRAPHY DESIGN DECISIONS -

**Chromatographic Resin:** \_\_\_\_\_

**Chemistry of Resin** (how does the resin separate the protein): \_\_\_\_\_

**Conditions for binding/separation** (think of buffer conditions to allow the protein to bind or separate, volume of load, other issues based on this specific chromatography) :

- Will the protein bind in low or high salt concentrations? (High / Low) \_\_\_\_\_
- Are there interfering compounds in the protein load that will block binding of the protein to the column (EDTA, imidazole, ammonium sulfate, ionic concentration). \_\_\_\_\_
- What is the concentration of salt currently in the sample? \_\_\_\_\_
- Is there a volume limit on the sample to load on the column?  
If yes what is the limit? \_\_\_\_\_
- Does the sample need to be concentrated or dialyzed? (Yes / No)
- How will the protein be eluted? (Step / gradient)
- What will the pH of the binding, equilibration and elution buffers need to be? \_\_\_\_\_
- What is the volume of your column? \_\_\_\_\_
- Gravity or Pump flow control? \_\_\_\_\_
- Will you wash weak bound proteins (Yes / No)
- What are the conditions to elute the protein  
- Gradient? From what to what concentration? \_\_\_\_\_  
- Isocratic? What conditions will you use? \_\_\_\_\_
- Flow rate \_\_\_\_\_

**Preparation of Column and Buffers:**

- Where is the resin? \_\_\_\_\_
- Do I save the resin and column or throw away \_\_\_\_\_
- Regenerating / Preparation of resin  
- high salt wash - use 10 ml per ml of resin \_\_\_\_\_  
- equilibration buffer - use 10 ml per ml of resin \_\_\_\_\_
- Equilibration / loading buffer (composition)  
- Volume needed (calculate column prep, load & wash vol.) \_\_\_\_\_
- Wash buffer (to remove weakly bound protein) composition \_\_\_\_\_
- Elution buffer composition \_\_\_\_\_
- High salt buffer composition \_\_\_\_\_
- Fraction Size \_\_\_\_\_



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**Equilibration buffer** (name of buffer should include the type of resin i.e. DEAE Equilib Buffer pH 8.0)

Buffer strength and pH \_\_\_\_\_

Other component(s) \_\_\_\_\_

Volume to prepare \_\_\_\_\_

*Calculations:*

**Wash buffer** (name of buffer should include the type of resin i.e. DEAE Wash Buffer pH 8.0)

Buffer strength and pH \_\_\_\_\_

Other component(s) \_\_\_\_\_

Volume to prepare \_\_\_\_\_

*Calculations:*

**Elution buffer** (name of buffer should include the type of resin i.e. DEAE Elution Buffer pH 8.0)

Buffer strength and pH \_\_\_\_\_

Other component(s) \_\_\_\_\_

Volume to prepare \_\_\_\_\_

*Calculations:*

**Wash buffer** (name of buffer should include the type of resin i.e. DEAE Wash Buffer pH 8.0)

Buffer strength and pH \_\_\_\_\_

Other component(s) \_\_\_\_\_

Volume to prepare \_\_\_\_\_

*Calculations:*

Method of detection of total protein : \_\_\_\_\_

Method of detection of MGH : \_\_\_\_\_