



Purification Table Instruction



Theory and Introduction: A purification table is used after a series of separation steps. This table, similar to the one you observed in the purification tutorial, is used to present the information about the yield and purity of the purification. There are only a couple of data needed to prepare the table:

- Volume of each step of the purification, starting from the lysates, to the final pooled sample.
- Total protein (mg) for each step.
- Units (amount) of protein being purified (typically done using the enzyme activity units).

The rest of the information for the table can be calculated from this data.

Example Purification Table

Fraction	Volume (ml)	Total Protein (mg)	Activity (units)	Total Activity (units * ml)	Specific Activity (units / mg)	Fold Purification	% Yield
Lysate	10.00	56.3	65897	658970	1170	1.0	100
DEAE	12.50	25.36	42845	535563	1689	1.4	81
Ni-Agarose	8.25	18.25	51481	424718	2820	2.4	64
S-200	4.30	3.75	48239	207428	12863	11.0	32
Reactive Blue	3.50	1.28	46578	163023	36389	31.1	25

Calculations -

1. **Units** - Normally, this is determined using an enzymatic assay. MGH has an enzyme, as one of the fusion partners, but we are determining the fluorescence of the protein as a function of the enzyme. **THIS MUST BE DONE AT THE SAME TIME.** You must thaw the fraction of your lysate, pooled samples and final purification sample and conduct a fluorescent assay at the same time. Different settings on the plate reader will give you a different result, thus it must be read at the same time using the same plate. Simply thaw the samples and place 100 μ l into a well. Return the samples back to the tube for later assay. The units of activity will thus be measured as MGH Fluorescence (relative fluorescent units). For specifics on how to determine enzymatic activity, go to the lab website for that handout.
2. **Total Activity** - this is a measure of how many units of protein you have in a sample. Simply multiply the activity in the sample by the total volume.
3. **Specific Activity** - specific activity is a way to measure how much of a measured protein (MGH in this case) there is with all of the other contaminating proteins. Divide the Activity by the mg of protein. The higher this value, the higher purity.
4. **Fold Purification** - Divide the specific activity of each fraction by the specific activity found in the lysates. This number changes depending on the protein you are working with. There is no good or bad value. However, this number along with the percent yield, indicates if a step was worthwhile or not. A poor fold purification with a low yield is a step to avoid in the future, while a high fold purification and high yield is a great thing...
5. **Percent Yield** - Calculate the percentage of the yield for each step using the total activity from the starting step.

Try setting up an excel spreadsheet using the formulas given above.