HOW TO USE THE AKTA:

EQUILIBRATION of Columns:
All columns should be equilibrated with atleast 1 column volume of the elution buffer before use. To equilibrate:
Go to File>Run> Rachel> method (either small column-200 1030 or large column-GF2001660 )
Set fraction size to 0ml, 0 ml flowing through the loop (you will wash the loop separately)

Save the wash result under Rachel>Washes>Column#

For proteins less than 10 mg:
use the S200 10/300 column with the 1ml Loop.

This column is 25 ml so it takes ~30 minutes to run. Concentrate the protein to 500ul. When loading the protein onto the column use the 1ml syringe and a 0.7mM injection needle (should be on or near the AKTA).

Use program S200 1030 located in Rachel's method folder. The max pressure of the column is 1.5 mPa, and the max flow rate is 1 ml/min. We typically run the column at ~0.75 ml/ml.
To start the Run:
Go to File>Run> Rachel> method (either small column-200 1030 or large column-GF2001660 )

During the methods section please make sure that the following parameters are assigned:
3 ml of buffer should be flowed through the loop after injection of the protein onto the column
Each fraction should be 0.5 ml
The run should last 1.25 column volumes

For Proteins over 10 mg or 2G12
use the S200 16/60 column with the 2ml Loop.

This column is 120 ml so it takes ~2 hours to run. Concentrate the protein to 1ml. When loading the protein onto the column use the 3ml syringe and a 0.7mM injection needle (should be on or near the AKTA).

Use program GF200 1660located in Rachel's method folder. The max pressure of the column is 1.5 mPa, and the max flow rate is 1 ml/min. We typically run the column at ~0.75 ml/ml.
To start the Run:

Go to File>Run> Rachel> method (either small column-200 1030 or large column-GF2001660 )

During the methods section please make sure that the following parameters are assigned:
8 ml of buffer should be flowed through the loop after injection of the protein onto the column
Each fraction should be 0.75 ml
The run should last 1.25 column volumes

NOTE ON BUFFERS:
Elute all* proteins on the SEC into TBS (20mM Tris pH 7.4 or 8, 150 mM NaCl, 0.02% NaN3)

*only exception is 2G12 D2 (WT, Xencor, or LALA) myc constructs.

Once purified collect the fractions under the peak (if there are multiple peaks as Anthony to help you decide which column to pick) and concentrate to atleast 1 mg/ml if possible. Try to concentrate it enough to fill 1 1.5ml eppendorf tube and spec on the nano drop.

To calculate the actual concentration of the protein:

Concentration = abs 280/extinction coefficient X molecular weight*

*most MW and e- coefficients are located in the google documents file current ei proteins.

Alternatively, you can spec the protein on the nanodrop and give the protein to Pri who can calculate the actual concentration.

Once labeled, and concentration determined please give to Pri for final storage.