Protocol for FPLC

Histrap HP 5mL:

1. Execute 'Pumpwash' to wash the column with 20% ethanol and ddH2O.
2. Assemble the Histrap HP(5mL) on the ÄKTAFPLC.
3. Put the pump A into ddH2O. Set the flow rate at 5ml/min to equilibrate the column with at least 5 column volumes of ddH2O.
4. Put the pump A into binding buffer. Set the flow rate at 5ml/min to equilibrate the column with at least 5 column volumes of binding buffer.
5. Put the pump A into the pretreated sample. Set the flow rate at 1ml/min to apply the pretreated sample.
6. Put the pump A into bind buffer. Set the flow rate at 3ml/min to wash the column until the absorbance reaches a steady baseline.
7. Put the pump A into bind buffer and pump B into elution buffer. Elute with elution buffer using a linear gradient.
8. Wash the column with at least 5 column volumes of elution buffer. Collect the eluents.
9. Take turns to wash the column with binding buffer and elution buffer until the absorbance reaches a steady baseline. Equilibrate the column with binding buffer.
10. Put the pump A into ddH2O. Equilibrate the column with at 10 column volumes of ddH2O. Disassemble the column.

Superdex 200:

1. Execute 'Pumpwash' to wash the column with 20% ethanol and ddH2O.
2. Assemble the Superdex 200 on the ÄKTAFPLC.
3. Put the pump A into ddH2O. Set the flow rate at 1ml/min to equilibrate the column with at 1.5 column volumes of ddH2O.
4. Put the pump A into buffer A. Set the flow rate at 1ml/min to equilibrate the column with at 1.5 column volumes of buffer A.
5. Wash the loop with ddH2O and buffer A.
6. Inject the pretreated sample into the loop and set the flow rate at 1ml/min to apply it.
7. Elute with buffer A and collect the eluents.
8. Wash the column with buffer A until the conduct reach a steady baseline.
9. Equilibrate the column with at 1.5 column volumes of ddH2O and 20% ethanol.
10. Disassemble the Superdex 200.