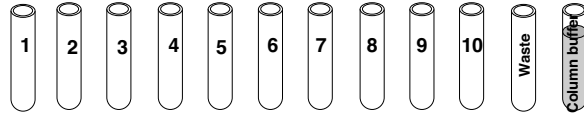
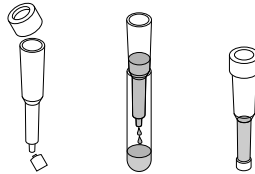


### Laboratory Protocol

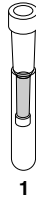
1. Place the 12 collection tubes in your test tube rack. Label 10 collection tubes sequentially from 1 to 10. Label the last two tubes “waste” and “column buffer”. Label either the tubes or the rack with your name and laboratory period.
2. Pipet 4 ml of Column Buffer into the tube labeled column buffer.



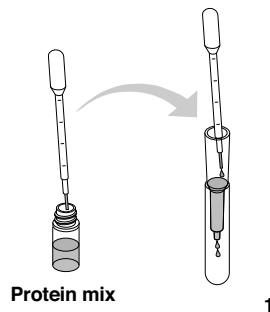
3. Remove the cap and snap off the end of the Poly-Prep sizing column. Drain all of the buffer into the “waste” collection tube. Cap the bottom of the column with the column end cap.



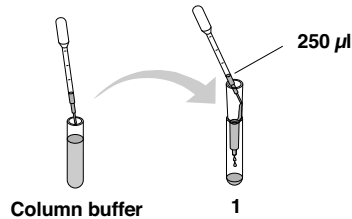
4. Gently place the column onto collection tube 1 (Do not jam the column tightly into the collection tubes—the column will not flow). You are now ready to load the protein sample onto the column.



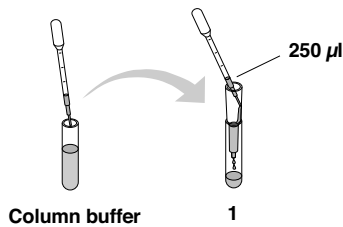
5. Remove the end cap from the column. Observe the top of the column bed; all of the buffer should have drained from the column. This is best observed by looking directly over the column—the “grainy” appearance of the column beads should be visible. Carefully load one drop of protein mix onto the top of the column bed. The pipette should be inserted into the column and the drop should be loaded just above the top of the column so that it minimally disturbs the column bed.



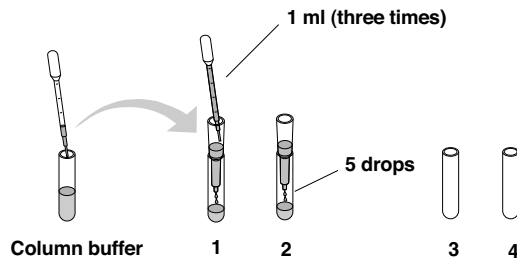
6. Allow the protein mix to enter the column bed. This is best observed by looking directly over the column. Carefully add 250  $\mu$ l of column buffer to the top of the column. This is best done by inserting the pipet tip into the column so that it rests just above the column bed. Carefully let the buffer run down the side of the tube and onto the top of the bed. Begin to collect drops into tube 1.



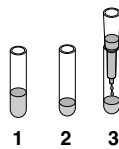
7. When all of the liquid has drained from the column, add another 250  $\mu$ l of column buffer to the top of the column. Add the buffer as before, by placing the pipette just above the top of the column and letting the buffer run down the side of the tube. Continue to collect drops into tube 1.



8. When all of the liquid has drained from the column, add 3 ml of column buffer to the top of the column. This can be done by adding 1 ml from the pipette three times. At this time the protein mix has entered the column far enough so that slight disturbances to the column bed will not affect the separation. Transfer the column to tube 2 and begin to count the drops that enter into each tube. Collect 5 drops of buffer into tube 2.



9. When 5 drops have been collected into tube 2, transfer the column onto tube 3. Collect 5 drops of buffer into each collection tube. When 5 drops have been collected into a tube, lift it off and transfer it to the next tube.



10. Continue collecting 5 drops into each tube. When you reach tube 10, collect a final 10 drops. Cap the column when finished collecting drops. Store your samples and column according to your teachers instructions.

