

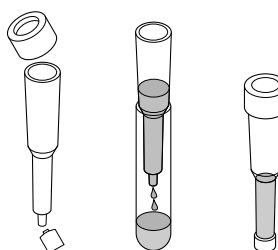
# Laboratory Quick Guide

## Size Exclusion Chromatography Kit

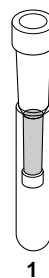
1. Obtain 12 collection tubes and label ten sequentially from 1 to 10. Label the tubes with your name and laboratory period. Label the final two tubes “Waste” and “Column Buffer”. Using a clean pipette, transfer 4 ml of column buffer into the tube labeled “Column Buffer”.



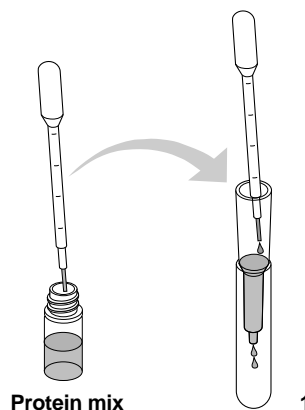
2. Remove the cap and snap off the end of the sizing column. Allow all of the buffer to drain into the waste tube. Observe the upper surface of the matrix and insure that all of the buffer has entered the column. Looking directly over and into the column, you should see the “grainy” appearance of the column matrix. Cap the bottom of the column.



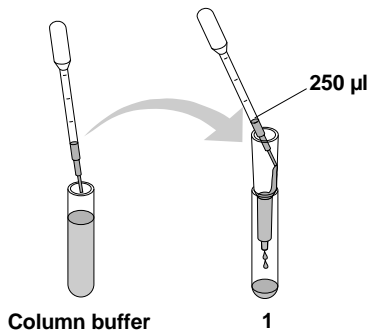
3. Carefully place the column onto tube 1. You are now ready to load (or the teacher may load) the protein sample onto the column.



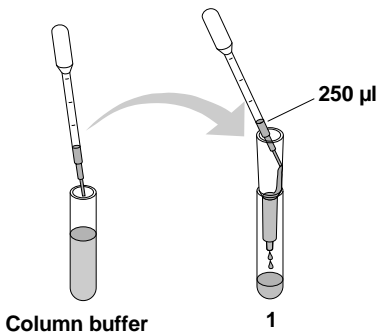
4. When you are ready to load the protein mix, uncap the column. It is important to uncap the column only when you are ready to load your protein—you do not want your column to run dry. Using a pipette, add one drop of protein mix onto the top of the column bed (your teacher may do the loading for you). 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.



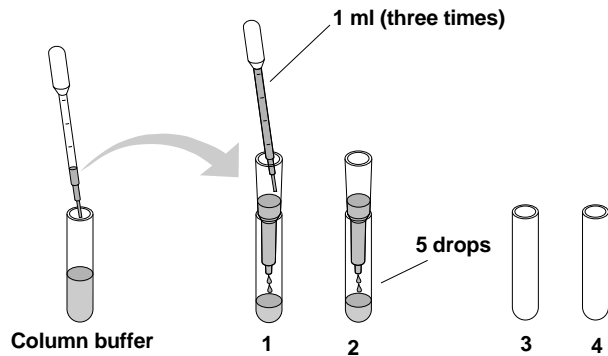
5. As soon as the drop of protein mix enters the column bed, carefully add 250  $\mu$ l of column buffer to the top of the column. This is best done by inserting the pipette tip into the column so that it rests just above surface of the column matrix. Carefully let the buffer run down the side of the tube and onto the top of the bed. (Note: The size separation will work best when the column bed is left undisturbed). Begin to collect drops into tube 1.



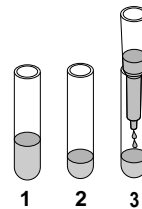
6. Add another 250  $\mu$ l of column buffer to the top of the column. Add the buffer as before, by placing the pipette just above the top of the column and letting the buffer run down the side of the tube. Continue to collect drops into tube 1.



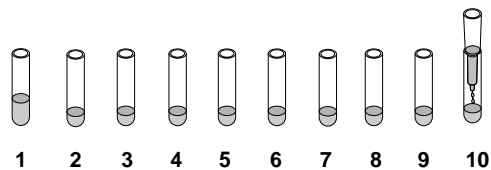
7. Add 3 ml of column buffer to the top of the column matrix. This can be done by adding 1 ml three times from the pipette. At this time the protein mix has entered the column far enough so that slight disturbances to the column bed will not affect the separation. Transfer the column to tube 2 and begin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



8. 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.



9. Continue collecting 5 drops into each tube. When you reach tube 10, collect a total of 10 drops. Cap the column and if your teacher instructs you to do so, parafilm or cover your fractions until the next laboratory period. Store the fractions in the refrigerator. Sketch your results.



**BIO-RAD**

**Bio-Rad  
Laboratories, Inc.**

*Life Science  
Group*

**Web site** [www.bio-rad.com](http://www.bio-rad.com) **USA** (800) 4BIORAD **Australia** 02 9914 2800 **Austria** (01)-877 89 01 **Belgium** 09-385 55 11 **Brazil** 55 21 2527 3454  
**Canada** (905) 712-2771 **China** (86-21) 63052255 **Czech Republic** + 420 2 41 43 05 32 **Denmark** 44 52 10 00 **Finland** 09 804 22 00  
**France** 01 47 95 69 65 **Germany** 089 318 84-0 **Hong Kong** 852-2789-3300 **Hungary** 36 1 455 8800 **India** (91-124)-6398112/113/114, 6450092/93  
**Israel** 03 951 4127 **Italy** 39 02 216091 **Japan** 03-5811-6270 **Korea** 82-2-3473-4460 **Latin America** 305-894-5950 **Mexico** 55-52-00-05-20  
**The Netherlands** 0318-540666 **New Zealand** 64 9 415 2280 **Norway** 23 38 41 30 **Poland** + 48 22 331 99 99 **Portugal** 351-21-472-7700  
**Russia** 7 095 721 1404 **Singapore** 65-6415 3188 **South Africa** 00 27 11 4428508 **Spain** 34 91 590 5200 **Sweden** 08 555 12700  
**Switzerland** 061 717-9555 **Taiwan** (8862) 2578-7189/2578-7241 **United Kingdom** 020 8328 2000