

Buffer-making exercise

In this exercise, you will work in groups of 3-4. Each group will make two buffer solutions, shown below, and then test the response of each buffer to addition of NaOH.

- 250 mL of 20 mM Tris, pH 7.2
- 250 mL of 200 mM Tris, pH 7.2

Tris is a very commonly used buffer in biotech labs. The company Sigma sells Tris under the trademarked name **Trizma**. It is sold in base and HCl forms; either will work for this application.

This exercise should be documented in your lab notebook.

1. Locate the chemical you will need in the chemical room, and note the FW of the solid.
2. Calculate the amount of solid you will need for each of the specified buffers.
3. For each buffer, fill an appropriately-sized beaker with dH₂O to about 80% of your final volume. (This volume need not be exact.) Label each beaker, identifying the contents (type of buffer and intended molarity and pH) and student names.
4. Weigh out your solid, and add it to the appropriate beaker. In your notebook, record the exact amount of each solid added to each buffer.
5. Add a magnetic stir bar to the beaker, place it on a stir plate next to a pH meter, and turn on the stirring to mix your solution. (If a stir plate is not available, obtain a spatula from the chemical room, and use that to stir the solution.)

Using the pH meter

6. Make sure that the pH meter is plugged in and working, that the attached probe is submerged in a tube labeled "storage buffer", and that the screen indicates that the meter is in pH mode (it will say pH above the big number on the screen).
7. Remove the electrode from the storage buffer and rinse the electrode using a dH₂O squirt bottle. Blot (don't rub) the electrode dry with a kimwipe.
8. Calibrate your pH meter. This is best done using a standard solution with indicated pH values. Since most of the measurements we will be doing in the lab are near pH 7, we will be calibrating to the pH7.0 standard.
 - Place the rinsed and dried tip of the electrode into the yellow pH 7 standard.
 - Wait for the pH meter to stabilize. You will know it is stabilized when an "S" appears on the screen.
 - Press "setup". The screen will go blank except for the flashing word "clear".

- Press “Enter”. This will erase the memory of the previous calibration. The screen should then look similar to what it did before.
 - Press “Standardize”. The meter will detect which standard you are using to calibrate and then calibrate to that pH.
 - Rinse and blot the electrode.
 - The meter is now calibrated.
9. Rinse and blot the electrode.
 10. Place the electrode in your buffer solution (you can use the stand to hold it in position), and the meter will begin to read your buffer. Once your group has calibrated the meter appropriately, you do not need to recalibrate for each reading in the same session. (It’s never a good idea to trust that someone else has done the calibration properly, though.)

Adjusting pH and completing your buffers

11. Note the initial pH of your solution in your notebook. Decide whether you need to add acid (HCl, to lower the pH), or base (NaOH, to raise the pH) to adjust the pH to 7.2. Remember to wear gloves when working with HCl and NaOH!
12. Make sure that your stir bar is on, or, if a stir plate is not available, make sure that you swirl or stir the solution thoroughly after each addition of acid or base to ensure you are getting a good reading. **Do not use the very fragile pH probe to stir!**
13. Start adding acid or base, as appropriate to adjust the pH of your solution to pH 7.2. A Pasteur pipet is often very useful for this. Your station may have a variety of concentrations of acid and base available. Use concentrated acid or base until you get to within 0.1 pH units of your target. Then, switch to a less concentrated form, to make sure you don’t overshoot the desired value.
14. Once you hit your desired pH, pour your solution into a graduated cylinder. Bring the volume up to the desired total. A dH₂O squirt bottle may be helpful here. Once you have the volume right, return your buffer to the beaker.
15. Complete your second buffer following the same procedure. (No recalibration of the pH meter is required.)

Testing buffer response to addition of NaOH

16. Once both buffers are made and at the proper pH (~7.2), test the response of each buffer to addition of a defined amount (1.0 mL) of 1 M NaOH. In your notebook, create a table like the one below, and fill in the data. Make sure you record the starting pH before adding your 1.0 mL of 1 M NaOH, and stir the solution well before recording the “after” pH.

Buffer	Starting pH (should be ~7.2)	pH after addition of 1.0 mL of 1 M NaOH	change in pH after addition
20 mM Tris			
200 mM Tris			

17. Clean up your bench area *and the weigh station*. **Rinse off the probe of the pH meter and return it to the storage buffer.** These are very important steps to ensure that these expensive meters are not ruined!!!
18. In the conclusions section of your notebook, address the following questions:
- Which buffer showed a smaller change in pH?
 - Why did that buffer show a smaller change in pH?

Micropipette Practice – Your test is in three weeks!

Perform a micropipetting practice session. Ask your instructor for a microcentrifuge tube containing 10 μL of dye. Cut a 1-2 inch section of parafilm and smooth it onto your bench. Using a micropipette tip, make ten small dents in the parafilm. Using a p10 micropipettor, pipet 1 μL of dye into each dent. Did you have enough for all 10 drops? Did you have some dye left over? Either of these results indicate pipette error.