

How to Make a Buffer

To prepare a buffer solution based upon an acid such as acetic acid or phosphoric acid add a strong base such as sodium hydroxide to a solution of the acidic form of the buffer until the desired pH is reached. To prepare a buffer solution based upon a base such as Tris or Imidazole add a strong acid such as HCl to a solution of the basic form of the buffer until the desired pH is reached. For preparation of a Tris buffer solution, the amount of Tris necessary to generate a given volume of buffer solution at a certain concentration is calculated i.e. 1 L of a 100 mM buffer solution would require 0.1 moles of Tris. The calculated amount of Tris is then dissolved in distilled water, the volume of water used should be about 80% of the given volume of buffer solution, i.e. 800 mL of water for the example above. A pH meter is then placed in the solution and the pH of the stirred solution is adjusted to the desired value with acid, usually 1 N or concentrated HCl for Tris. The solution is then topped off with distilled water to the final volume, 1 L for the example above. If small quantities of buffer solution are to be used it is sometimes desirable to make a bit extra due to the increased ease of making the solution, standard pH meters work best with a volume of greater than 10 mL. In addition, when making concentrated buffer solutions, especially for Tris, make sure the solution is close to room temperature before topping off with distilled water.