SOP: R007

Preparation of Buffer D for SDS-PAGE (0.5 M Tris-HCl, pH 6.8)

Materials and Reagents: (per 500 ml)

1. Graduated cylinder, 500 ml
2. Glass beaker, 500 ml
3. Stir bar
4. Magnetic stir plate
5. pH meter
6. Sterile 500 ml glass bottle
7. Milli-Q water
8. Tris base, electrophoresis grade, 30.28 g
9. Hydrochloric acid (HCl) 12 N

Protocol:

1. _____ Measure out and pour 400 ml of Milli-Q water into glass beaker with a stir bar in it and begin stirring.

2. _____ Slowly add Tris base to the water (over a period of several minutes)

3. _____ When all the Tris base is in solution, place washed pH meter probe in the solution. (The pH will be approximately 10.3).

4. _____ Begin adding 12 N HCl dropwise to the stirring solution (note 1).

5. _____ When the pH reaches the 7.2 to 7.4 range, let stir for 5 min to make certain the pH is the same throughout the solution, as well as to let it equilibrate to room temprature.

6. _____ Dilute some of the 12 N HCl to 6 N by adding approximately 5 ml of 12 N HCl to 5 ml of Milli-Q water (note 2).

7. _____ Add 6 N HCl dropwise to solution until the pH is 6.8. Let stir a couple more minutes to ensure the pH reading is stable.

8. _____ Pour solution into graduated cylinder and add enough Milli-Q water to bring the total volume to 500 ml.

9. _____ Transfer Buffer B to sterile 500 ml bottle.

Notes:

1. It will take quite a bit of HCl.
2. Always add acid to water, adding water to acid can cause the solution to flash.

References:

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. (London). 227:680-685.