**pCa Solution Protocol**

**Planning**

The process of making pCa solutions is long and extremely easy to mess up. At least two full days should be reserved, one for checking materials and labelling vials and one for mixing the solutions. There are many steps, most of which could render the solutions useless if a mistake were to be made. So take your time and keep everything neat and organized throughout the process.

Make sure you have everything needed for making the solutions several days before so that if anything is needed we can get it for you. This includes the chemicals, glassware, storage tubes and space in the freezer. Label all the cryogenic tubes before the day you will be making solutions as well, it takes a surprisingly long time!

**Materials**

Chemicals:

There are several different recipes available. Select the correct one for your application (talk to Ken if you're not sure) and check to make sure all chemicals are in stock. You should also check the purity values and the math producing the final weights needed before starting.

Equipment:

1L and 500ml beakers- 1 each

250ml and 500ml volumetric flasks – 1 each

50ml beakers – x12

100ml graduated cylinder

Pipettes and tips (300µl, 5ml, and 10ml)

Spatulas

Scale accurate to 0.0001g

pH meter and calibration solutions

At least 3 Ice buckets

Magnetic stir plate and bars

Cryogenic tubes (1.5ml and 5ml)

Boxes for cryogenic tubes

**Procedure**

1. Print out the recipe tables that fit your application

2. Set out weigh boats, spatulas, ready the scale and stir plate, and fill the Ice buckets

3. Partially fill the two large beakers with filtered DI water, just enough to dissolve the contents (about 1/3 of the final volume)

4. Label the beakers “pCa 4.5” and “pCa 9.0” and place them on the stir plate with bars.

5. Carefully weigh out chemicals one at a time and place them into the respective solutions. Rinse the weigh boat into the solution with a small amount of filtered DI each time. Make sure that you get ALL the chemical off.

6. Record the exact amount added and the lot number for each chemical as you use them.

a. This will eliminate any uncertainties if you make a mistake at some point.

7. When you are done weighing out the chemicals, calibrate the pH meter and fill the ice bucket with ice.

8. Cool the solutions to 15 degree celsius ( the experimental temperature) and check the initial pH.

a. the best way to do this is to place the beakers on ice for sometime and then back on the stir plate.

9. Add 4M KOH in small amounts until you reach proper pH and record the amount added

a. Check the final ionic strength using the procedure outlined below

10. Pour the solutions into their respective volumetric flasks and bring the volume up using filtered DI. Place the flasks in ice.

11. Label the small 50 mL beakers for the range of pCa solutions you will be making and arrange them in ice.

12. Using the Mixing Table, pipette first the 4.5 into all of the beakers, then the 9.0.

13. Make sure they are mixed well then pipette into the cryogenic vials

14. Store upright in a -20c freezer.

**Solution Checks**

The recipe is set up so that the solution should be slightly acidic when everything is added. KOH will need to be added to bring the pH up to 7.0. After adding the KOH it is important to check that the final ionic strength is close to 180mM. From the program we know how much KOH should be added to bring the ionic strength up to 180nM. For example, in a pCa 9.0 solution , 16mM of KOH used for pHing will bring the final ionic strength to 180nM. So, after adding KOH, total up the volume added and use the following formula to find the final ionic strength of KOH in the solution.

C1V1 = C2V2

Where C1 is the concentration of KOH added; V1 is the volume added; C2 is the final concentration of KOH; and V2 total volume of the solution. So if 600µl of 4M KOH was added to a recipe for 250mL, the formula would be:

4M \* 600µl = C2 \* 250mL

C2 = 9.6mM

This is within 10mM of the desired final ionic strength (16mM) of KOH, so nothing else needs to be done. If less than 6mM had been added while pHing then make up the difference using KCl. If more than 26mM had been added to the solution, You must assume something else was added incorrectly and start over from the beginning.

**Positive Control**

It is also important to check your final solutions by running an experiment where we know the Force and ktr values, eg rat soleus fiber. For example, use rat soleus muscles to collect 4-5 tension pCa curves to reproduce results published by Ken in [his 2002 paper](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1301901/).