

Protocol for making Rat Tail Tendon Type I Collagen Gels

You will need the following items on ice: 10x PBS (pH 7.4), acid solubilized rat tail tendon collagen stock solution, water, 10 N NaOH, and a tube for mixing ingredients. These items should be sterile if it is necessary to keep the tissues for a while. Collagen stock solutions are typically obtained from BD Biosciences (high concentration rat tail tendon collagen I, cat. # 354249, 100 mg is \$132).

Mix these ingredients in the following order on ice, for a 1-4 mg/ml collagen gel that has a volume of X ul:

1. $X/10$ ul of 10x PBS
2. water
3. Y ul of Z mg/ml collagen stock solution. For a desired gel concentration, you can figure out how much stock solution you need.
4. Make sure the above three ingredients are well mixed by vortexing for a few seconds. Then place the mixture back on ice.
5. Measure pH with pH strip or pH electrode. Adjust with 10 N NaOH if necessary.
6. Simply pipette the collagen mixture into the wells or dishes in which you wish the gel to polymerize. Put the gels in 37 degree Celsius incubators for quick polymerization (about 30 minutes) and thin fibers. Put the gels in 4 degrees Celsius refrigerator or cold room for 48 hours for thicker fibers. You can also polymerize at other temperatures between 4-42 degrees Celsius. Gels polymerize more slowly at colder temperatures; 48 hours is a time I use to ensure complete polymerization. Ensure no evaporation can occur from the samples by wrapping with parafilm if necessary. After the appropriate temperature incubations, the gels should be firm and somewhat cloudy.

Example:

For a 4 mg/ml collagen gel with volume 500 ul:

1. 50 ul 10x PBS
2. 200 ul water.
3. 250 ul 8 mg/ml collagen stock solution
4. vortex
5. check pH, adjust if necessary. Collagen will polymerize at about pH 6.0 – 8.0. Gels polymerized at lower pH are weaker.
6. pipette into desired wells
7. Incubate at desired temperatures (4 – 42 degrees Celsius). Gels polymerized at lower temperature (especially less than 24 degrees C) are weaker and have thicker fibers.
8. When I vary the temperature of polymerization I keep the pH constant at 7.4.
9. This will produce a gel with $I \sim 0.4$. If you want a larger I, use more 10x PBS.