# User Protocol: Collagenase Degradation of CollaFibR™ for Cell Recovery

**Equipment and materials needed:**

- Humidified incubator
- Biological safety cabinet (BSC)
- Water bath
- Cell culture plate containing CollaFibR™ constructs
- Pre-warmed sterile dPBS

**Solubilizing Collagenase IV:**

1. Check the lot specific number of enzymatic units (U) per mg.
2. Calculate number of mg (or mL) required to make a 10,000 U/mL stock of collagenase IV.
3. Solubilize collagenase IV in 1x PBS, mix thoroughly by inverting and shaking.
4. Sterile filter collagenase IV with 0.2 µM PES filter.
5. Aliquot 1 mL into labelled 1.5 mL Eppendorf tubes and store at -20˚C in an opaque box.

**Degrading collagen fibers with collagenase IV:**

1. Collect sample of fibers with or without cells, by aspirating the entire volume from the cell culture plate.
2. Spin at 130 x g for 6 minutes at room temperature which can increase to 300 x g if loose pellet is formed.
3. Remove supernatant and resuspend in 500 µL; 10 µL collagenase IV stock + 490 µL dPBS.
4. Incubate at 37˚C (and 5% CO2 if cells were in sample) for 10-30 minutes.

**If no cells were in sample – protocol is complete and fibers should be digested completely; if cells were used, proceed to step 5.**

5. Spin at 130 x g for 6 minutes at room temperature.
6. Remove supernatant and resuspend pellet in a small volume of DPBS. The pellet size will dictate – smaller pellets receive smaller volumes but use at least 10 µL.
7. Collect a small sample and mix 50:50 with trypan blue for counting or just add 10 µL trypan blue to the sample if the pellet was resuspended in only 10 µL.