# COLLAGEN COATING OR EMBEDDING
## WITH DAVID DANKORT

**IMPORTANT NOTE:** Keep collagen cold <4°C

Reagents: Collagen (Vitrogen from Cohesion labs [cat# PC0701] –100ml)
Sterile 10xPBS or 10x serum free media
Sterile 0.1N HCl
Sterile 0.1N NaOH
pH paper [4-10]

***Making neutralized, isotonic Collagen Solution***
*<all steps on ice unless stated otherwise>*

1. Mix 8 mL chilled Vitrogen Collagen
   1 mL 10X PBS solution or 10X serum free media
   1 mL 0.1 M NaOH and mix.
2. Adjust the pH to 7.4 ± 0.2 by the addition of a few drops of 0.1M HCl or 0.1 M NaOH. Monitor with pH paper or phenol red (@0.005 mg/mL).
3. The neutralized, isotonic Collagen can be stored at 4°-6°C for several hours prior to gelation.

***Gelation of neutralized, isotonic Collagen solutions.***

1. Collagen gelation (fibrillogenesis) is initiated by warming neutralized solution to 37°C. Gelation occurs more rapidly in the absence of $CO_2$ – For best results, allow a minimum of 60 minutes for gelation to occur.
2. Cells can be dispersed on collagen gels, sandwiched between collagen gels or suspended in collagen gels by mixing them with the neutralized Vitrogen Collagen solution prior to gelation.

***Coating Dishes-****Preparation of* ***Fibrillar*** *Collagen Films for Covering Cell Culture Surfaces*

1. Prepare neutralized, isotonic Collagen solution as above.
2. Cover surface with this solution to a depth 1-2mm (1-2 ML for a 35mm cell culture dish).
3. Incubate for approximately 60 minutes at 37°C to promote gelation.
4. Leave dish uncovered in laminar flow hood overnight or until dry.
5. Rinse film with sterile H2O in order to remove salts and rehydrate film.
6. Film can be used immediately for cell culture or allowed to dry again and be stored for future use.

***Coating Dishes-****Preparation of Collagen* ***Monomeric*** *Coatings*

1. Dilute Collagen with 0.01N HCI or 0.05M acetic acid prior to coating inside the dish or well.
2. Leave the dish uncovered in a laminar flow hood overnight to allow for normal evaporation.
3. Rinse dish with sterile PBS solution or media to remove residual acid and rehydrate collagen prior to use.
4. Collagen coatings prepared in this manner are nonfibrillar in nature and thus can be distinguished from the fibrillar collagen preparations described above.