

# Generation of Collagen: Matrigel mixed Gel

## 1) Neutralize collagen:

On ice, mix:

0.5 ml Collagen Type I (Vitrogen, bovine dermal collagen, from Cohesion Technologies, [www.cohesiontech.com](http://www.cohesiontech.com), at 2.9-3.2 mg/ml),  
62.5  $\mu$ l 10X PBS (sterile filtered),  
62.5  $\mu$ l 0.1 M NaOH (sterile filtered).

Bring the pH of the solution to 7.5 with approximately 10  $\mu$ l 0.1 N HCl.

## 2) Mix with Matrigel

On ice, mix the neutralized collagen I and growth factor reduced Matrigel<sup>TM</sup> such that the final collagen concentration is 1.6 mg/ml\*

(For example, if the lot of collagen is 2.9 mg/ml and you want to keep final concentration at 1.6 mg/ml, use 45% Matrigel<sup>TM</sup> and 55% Collagen).

\*In practice, we routinely just mix the neutralized collagen I and EHS 1:1 for an approximate 50/50 mix.

## 3) Make bed of mixed gel in chamber slides

Spread 50ul per well in an 8-well chamber slide.

Work as quickly as possible since collagen tends to solidify even on ice in a short time.

Incubate for 30-60 minutes at 37°C. Change medium as described before.