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 μl 10X PBS (sterile filtered),
62.5 μl 0.1 M NaOH (sterile filtered).

Bring the pH of the solution to 7.5 with approximately 10 μl 0.1 N HCl.

2) **Mix with Matrigel**

On ice, mix the neutralized collagen I and growth factor reduced Matrigel™ 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™ 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 50μl 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.