

## Col I Kit Reconstitution Protocol

### Kit Components

| Item                           | Quantity | Storage                      |
|--------------------------------|----------|------------------------------|
| CELLINK Col I Liquid - Sterile | 5 mg/mL  | -20 °C, protected from light |

### Materials not included

| Item               | Quantity |
|--------------------|----------|
| Stir Bar – Sterile | 1        |
| Sterile 10 X PBS   | 10 mL    |
| Sterile DI Water   | 10 mL    |
| Sterile 1 N NaOH   | 10 mL    |

### Protocol for Casting

1. Record the desired final volume of the ink ( $V_{INK}$ ).
2. Record the desired final collagen concentration ( $C_{Fconc}$ ).
3. Maintain the vial of Col I stock solution on ice to keep cool.
4. Prepare a neutralization solution for the collagen based on the following calculations:
  - a. Volume of ColIMA Stock Solution ( $V_{CoIMA}$ ) =  $\frac{C_{Fconc} \times V_{INK}}{C_{Conc}}$
  - b. Volume of 10X PBS ( $V_{PBS}$ ) =  $\frac{V_{INK}}{10}$
  - c. Volume of 1N NaOH ( $V_{NaOH}$ ) =  $V_{CoIMA} \times 0.023$
  - d. Volume of DI water ( $V_{DI}$ ) =  $V_{INK} - V_{CoIMA} - V_{PBS} - V_{NaOH}$
  - e. Mix the volumes from steps b, c and d. This is the neutralization solution ( $V_{NS}$ ).
  - f. Cool the neutralization solution on ice for 10 minutes.
  - g. Transfer the  $V_{CoIMA}$  to the tube containing the  $V_{NS}$ , gently mix via pipetting.
5. Cast structure and warm to 37 °C to induce gelation.