

## Product Information

Decellularized ECM bioink precursor, suitable for 3D bioprinting applications

Protocol for Catalog No. [906867](#), [906875](#), and [906883](#)

## Introduction

Decellularized ECM bioink precursor is an extracellular matrix (ECM) derived three-dimensional (3D) printable ink material. The decellularized ECM (dECM) is a biomaterial consisting of both structural and functional biomolecules, such as collagen, glycosaminoglycans and glycoproteins. Decellularized ECM bioink precursors have different characteristics depending on the origin of tissues (skin, bone, cartilage), thus providing optimized environments for cellular activities that are tissue specific.

## Disclaimer

Decellularized ECM bioink precursor is for research use only; not suitable for human, animal, or other use. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Specifications

<b>Storage</b>	Store decellularized ECM bioink precursor at -20 °C for up to 12 months.
<b>Stability</b>	Refer to the expiration date on the batch-specific Certificate of Analysis.

## Materials

### Materials supplied

Decellularized ECM bioink precursor lyophilized solid 100 mg

### Materials required, but not supplied

- 0.5M acetic acid
- 10× Minimum Essential Medium (MEM) (Cat. No. [M0275](#))
- HEPES (Prod No. H4034)
- Sodium bicarbonate (NaHCO<sub>3</sub>)
- Deionized or Distilled water
- Sodium hydroxide (NaOH)
- Cultured cells ([visit our website for an up-to-date list of cell types](#))
- Appropriate cell culture medium
- PBS

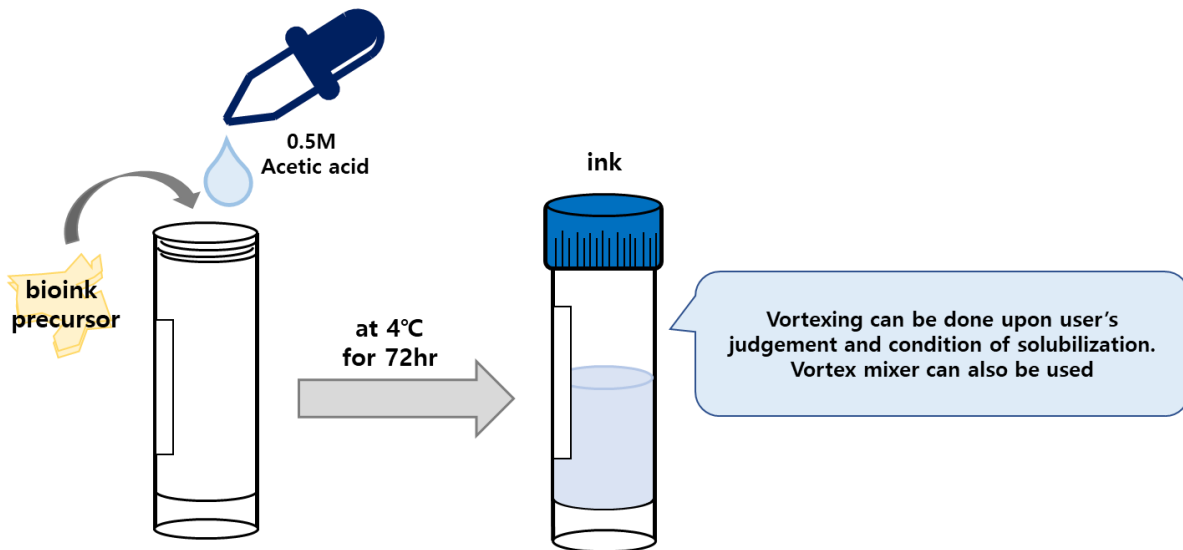
- Sterile tube for reagent preparation
- Positive displacement pipette is recommended)
- Sterile pipette tips for transferring bioink
- Sterile printing cartridge, piston, and nozzle/needle for 3D printing
- Extrusion-based 3D bioprinter
- Petri-dish, scraper
- Centrifuge
- pH indicator

## Before you start: Important tips for optimal bioprinting results

1. The product needs to be processed on a clean bench to maintain the sterility of the product
2. All processes should be done at 2-8 °C (ice bath or fridge) to prevent gelation of the solubilized bioink precursor.
3. The solubilized bioink should be prepared and used immediately

## Procedure

### A. Solubilization process



1. Prepare 0.5M acetic acid and “decellularized ECM bioink precursor” on ice.
2. Place the “decellularized ECM bioink precursor” in a glass vial and add 0.5M acetic acid to the desired concentration.

**Recommendation:** at least 3% (30 mg/ml) for printing

3. Keep the sample at 2-8 °C (in a fridge) for 72 hours for solubilization.

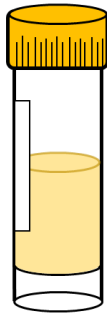
**Note** – Vortexing ink every 24 hours during solubilization process

### B. Reagent preparation

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

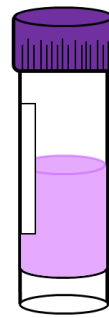
**Millipore  
Sigma**

### 10x MEM



Recommendation  
Sigma-Aldrich  
(Catalog #M0275)

### RB buffer



NaHCO<sub>3</sub>  
HEPES  
Distilled water  
NaOH

✓ It is recommended to use RB buffer within 7 days after preparation.

## 1. Preparation of resuspension buffer (RB) Reagent

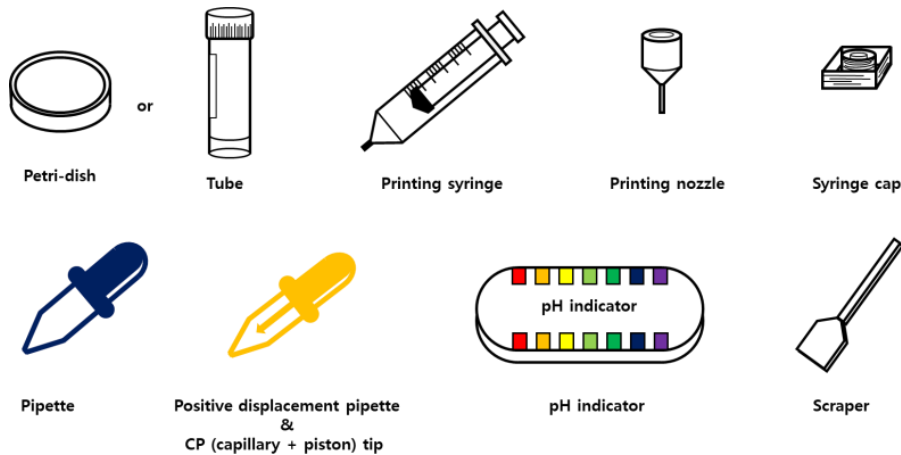
RB composition-

	Skin <a href="#">906867</a>	Bone <a href="#">906883</a>	Cartilage <a href="#">906875</a>
NaHCO <sub>3</sub> (g)	0.22	0.22	0.22
HEPES (g)	0.48	0.48	0.48
Distilled water (ml)	10	10	10
NaOH (g)	1.40	1.70	1.70

**Note** – Use RB buffer within 7 days after preparation.

## C. Bioink preparation procedure

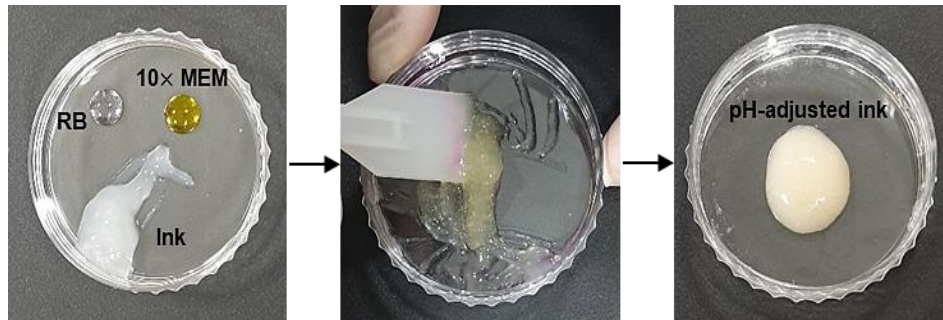
### Supplies needed



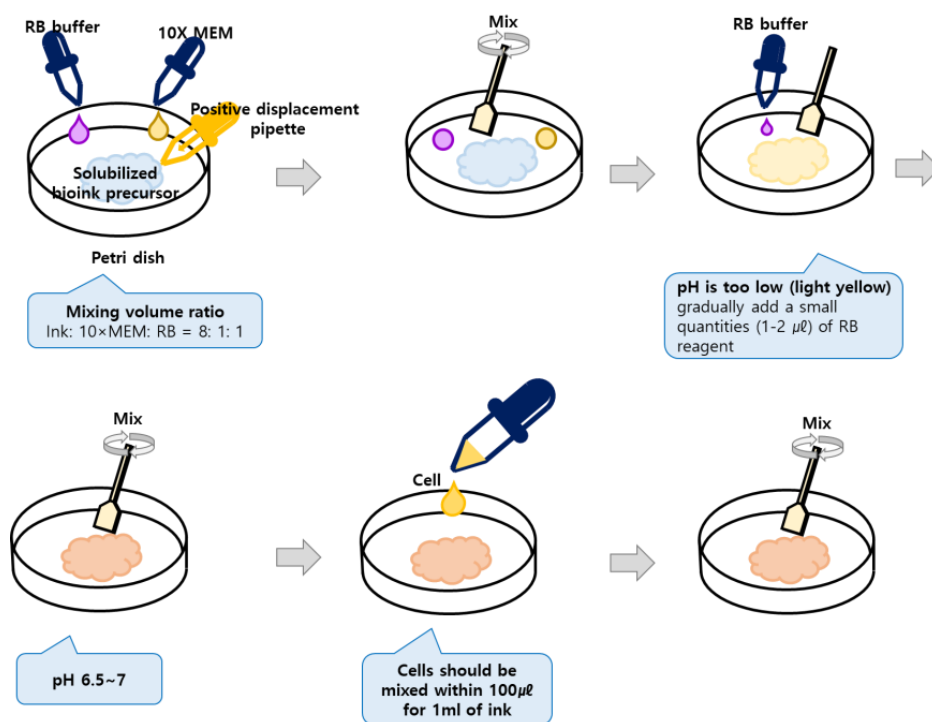
1. Place RB, 10xMEM (Minimum Essential Medium, Cat. No. [M0275](#)) and solubilized bioink precursor on ice to cool down for ~10 mins.
2. Mix solubilized bioink precursor, 10xMEM and RB at volume ratio of 8 : 1 : 1. The pH of the final ink should be between 6.5–7.0.

## Note

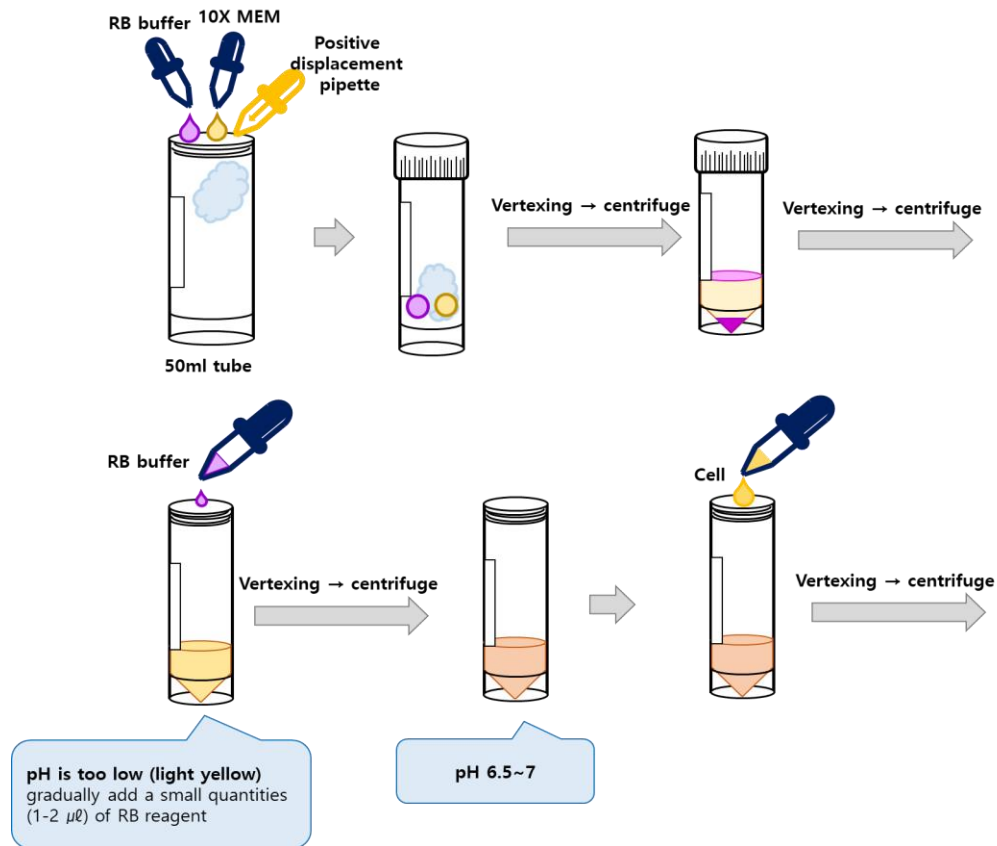
- Prior to bioink preparation, centrifuge the solubilized bioink precursor to remove residue bubbles. (Centrifugation condition: 2500 rpm, 5min)
- For adjusting pH, use at least 800  $\mu\text{l}$  of ink and measure the ink volume as accurate as possible.
- The color of final bioink should be light orange.
- If pH of the bioink is too low (indicated by light yellow or yellow color), add small quantities (1-2  $\mu\text{l}$ ) of RB reagent each time and gradually adjust it to the ideal pH range.



## Prepare in petri dish

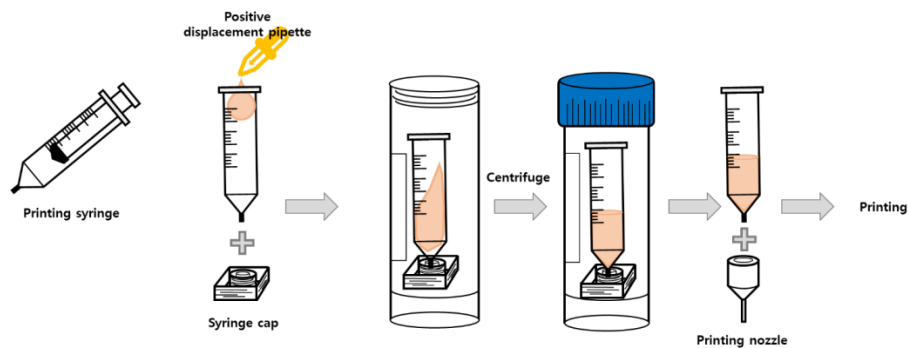


## Prepare in 50mL tube



3. Resuspend the cell pellet at the desired cell density with the bioink solution by gently pipetting up and down. Maximum 100 $\mu\text{l}$  cell suspension can be mixed with 1ml of bioink.

## D. Printing procedure



1. Transfer ink or solubilized bioink precursor to a printing cartridge.  
**Note** - Centrifugation can be used for removing residual bubbles  
(Recommended condition: at 2,500rpm for 5 minutes at 4 °C)
2. Start printing process using bioink, and then incubate the printed construct for 40~60 minutes without any solutions (ex. culture medium) in an incubator (at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>) for gelation.  
**Note** - Recommending printing temperature: Below 15°C within 3 hours / Room temperature within 30 min

**Example**

*Printer: Cellink BIO X™ or Cellink INKREDIBLE™ printer*

*Temperature: 15 °C*

*Flow rate (speed): 10 mm/s*

*Nozzle: 22G TT tapered needle*

*Pressure: 50–60 kPa for Cartilage and Bone bioinks*

**E. Culture cells**

Culture the bioprinted tissue with the appropriate cell culture medium following standard tissue culture procedures.

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