Directions For Use

Collink.3D™ 50L
Recombinant Human Type I Collagen Methacrylamide
Catalog Number W10204

1. Product Description

Collink.3D™ 50L is a recombinant human type I collagen (rhCollagen) methacrylamide lyophilized, to be used as a curable base bioink material for biofabrication applications, including 3D bioprinting. The product is produced from purified rhCollagen extracted from genetically engineered tobacco plants, that was chemically modified to generate curable human collagen with approximately 50% of the primary amines converted into methacrylamides. The material is supplied in a dry form and can be reconstituted and formulated with other components such as synthetic acryl/thiol monomers and/or polymers, different types of photoinitiators, photoblockers and biological additives. Thereafter, formulations can be crosslinked to form hydrogels with diverse properties for use in a variety of applications.

Collink.3D™ 50L is compatible with major 3D bioprinting technologies, i.e., extrusion, inkjet, photolithography and laser-induced forward transfer (LIFT).

Collink.3D™ 50L product is characterized by high flexibility to incorporate into bioinks with no limitation of a buffer type or concentration, as well as easy hand-ability.

2. Indications for Use

Collink.3D™ 50L is a bioink platform material for biofabrication of scaffolds, tissues and organs for 3D modeling and implantation.

Biofabricated constructs composed of Collink.3D™ 50L can be used in a wide range of tissue model applications, including drug discovery, drug screening, disease modeling and tissue testing. In addition, it can be used for spheroids/organoids biofabrication, as well as for the research, development and manufacture of implantable scaffolds, tissues, and organs with complex architectures, to meet specific properties.

Note that Collink.3D™ 50L is for R&D use only and is not intended for human use.

3. Storage

The product is shipped in temperature-controlled packaging. Upon receipt, store the product at 2-8 °C.

Do not freeze the product.

Protect the product from light.

4. Directions For Use

4.1 Operation conditions

- Prior to use, it is recommended to equilibrate the product to room temperature.
- Avoid heating the pre-cured product above 24 °C.
- Protect the material from light exposure.
- Aseptic practice is recommended.
- Unlike tissue-extracted collagen, Collink.3D™ 50L does not form a hydrogel upon heating, and therefore, cooling during the printing process is not required.
- Avoid heating the product during the curing/printing process as it may affect product integrity and performance.

4.2 Reconstitution

- Reconstitution can be performed with a variety of solvents, including weak acids (low concentration HCl and acetic acid), buffers (phosphate buffer, commercial Phosphate Buffered Saline (PBS)), cell media and water.

  NOTE: Chilling of collagen and related working solutions is not required during preparation.

  It is recommended to solubilize the lyophilized collagen methacrylamide with 10 mM HCl at concentrations up to 15 mg/mL.

1. Add volume of 10 mM HCl (see Table 1) to the lyophilized collagen methacrylamide to achieve the desired concentration.
2. Mix on a shaker table or rotator plate at room temperature until fully solubilized or overnight. The expected mixing time to reach 10 mg/mL solution in 10 mM HCl is 3 hours.
   NOTE: The higher concentrations of collagen will take longer to solubilize.
   Avoid the formation of air bubbles as possible. If formed, eliminate by spin-down.
3. In case reconstitution of only a part of the dry cake is desired, refer first to the product CoA (Dry weight attribute).
   Example: If the desired quantity of collagen is 50 mg, and the indicated averaged dry weight in batch CoA is 108 mg (which is 8% higher than the supplied quantity), then 54 mg shall be weighed.
4. To achieve a final pH of 7.0 to 7.4, follow the guidelines section 4.4.

Table 1: *Collink.3D 50L reconstitution volumes#*

Desired Collink.3D 50L concentration	Volume of 10 mM HCl#
3 mg/mL	33.3 mL
6 mg/mL	16.7 mL
8 mg/mL	12.5 mL
10 mg/mL	10.0 mL
12 mg/mL	8.3 mL

#Upon reconstitution of the entire bottle.

4.3 Dilution after reconstitution

Similar to reconstitution, further dilution after reconstitution can be performed with a variety of solvents (see examples in section 4.2).

Note that changing the pH and salt concentrations can lead to fibrillogenesis/de-fibrillogenesis, which may affect formulation opacity and viscosity.

Due to material viscosity, it is recommended to dilute by weight (assume a density of 1.0 g/cm3), rather than by volume. If dilution by volume is preferred, use positive displacement pipettes compatible with viscous liquids only.

Dilution in 10 mM HCl is recommended. For a neutral pH, refer to section 4.4.

Recommended working concentrations in bioink formulations: 3 mg/mL - 10 mg/mL, depending on formulation composition and the required properties (see Table 2).

After the addition of the diluent, shake, roll or mix at room temperature for 1-2 hours.

NOTE: Avoid the formation of air bubbles. If formed, eliminate by spin-down.

NOTE: Vortex is not recommended at any point (except for neutralization, see section 4.4).

Dilution examples, calculated for 1 g Collink.3D™ 50L reconstituted solution at an initial concentration of 15 mg/mL are provided in Table 1.

Table 2: Dilution of 1 g Collink.3D™ 50L solution with 10 mM HCl

10 mM HCl [mL]	Final concentration [mg/mL]	Final solution amount [g]
0.5	10	1.5
0.875	8	1.875
1.5	6	2.5
4.0	3	5.0

In the case of dilution with PBS, initial neutralization is recommended (see section 4.4).

4.4 Neutralization

Product neutralization to reach a physiological level is essential when conducting in-vitro experiments.

Employ aseptic techniques in cell culture hood if aseptic collagen is required.

Collink.3D™ 50L solution (after reconstitution, see section 4.2) can be neutralized by adding concentrated NaOH and 10X PBS targeting neutralized solution containing 15 mM NaOH and 1X PBS.

NOTE: Use freshly neutralized Collink.3D™ 50L solution (up to 2 days storage at 2-8 °C). It is not recommended to prepare and store a solution of neutralized Collink.3D™ 50L longer than the indicated period.

5. Weigh Collink.3D™ 50L solution (after reconstitution) into a light-protected tube.
6. Add concentrated NaOH (1N-0.1N, depending on the desired volume), to a final concentration of 15 mM.
7. Mix gently using a short vortex (mild conditions), until the entire solution appears opaque and viscous.
8. Add 10X PBS, in Collink.3D™ 50L/NaOH solution to 10X PBS ratio of 9:1 (see examples for neutralization in Table 3).
9. Perform short vortex (mild conditions). The solution will appear opaque (due to fibrillogenesis), and viscosity will increase.
10. Mix at room temperature for 1-2 hours until the solution becomes clear and homogenous. Avoid the formation of air bubbles. If formed, remove by spin-down.

11. Measure pH (pH test paper may be adequate with small volumes) to ensure neutral pH of 7.0-7.4. Adjust pH by titration if required.
12. If further dilution is required, first calculate the solution concentration after neutralization. Then, adjust the solution concentration to the desired concentration by diluting with 1X PBS (see section 4.3), taking into consideration the photoinitiator volume and other components to be added at a later stage.

Examples for neutralized solutions using 1 N NaOH are provided in Table 3.

Table 3: Reconstituted Collink.3D™ 50L neutralization ratios

Reconstituted Collink.3D™ 50L [g]	NaOH 1 N* [mL]	10X DPBS [mL]	Total [g]
1	15.2	102	1.12
2	30.5	203	2.23
3	45.7	305	3.35
4	60.9	406	4.47
5	76.1	508	5.58

If less than 1 g Collink.3D™ 50L solution is prepared, it is recommended to use lower NaOH stock concentrations, i.e., 0.5 N or 0.1 N.

4.5 Photo-crosslinking

Collink.3D™ 50L after reconstitution can be crosslinked at a wide range of pH values, i.e., from neutral (pH 7-8) to acidic (pH 2-3).

A minimum concentration of 3 mg/mL is recommended for the biofabrication of photo-crosslinked hydrogels, when no additional photocurable ingredients are considered.

NOTE: Reconstituted Collink.3D™ 50L will not form a physically crosslinked gel upon heating. Hydrogels can only be achieved by chemical crosslinking. Avoid heating Collink.3D™ 50L (before and/or after reconstitution) above room temperature before crosslinking.

The curing conditions (i.e., duration, distance, intensity) should be optimized to the photoinitiator type/ concentration, light source and the desired properties of the final construct.

Using a fresh photoinitiator stock solution is recommended (or up to 1-week storage at room temperature, in a sealed container).

1. Add the photoinitiator stock solution volume to the reconstituted Collink.3D™ 50L working solution (see reconstitution instruction in section 4.2 and dilution instructions in section 4.3), to reach the desired final concentration of the photoinitiator.
2. Mix thoroughly at room temperature for 2 hours, by shaking/stirring/rolling the vessel or tube. Keep the solution protected from light.
3. Before photocuring/3D-bioprinting, perform spin-down to remove air bubbles.

After the addition of a photoinitiator, Collink.3D™ 50L solution can be stored at 2-8 °C, light protected, for up to 2 days.

Examples of photoinitiators with their respective concentrations and required light source wavelengths are listed in Table 4.

Table 4: Examples of photoinitiators for photocrosslinking reaction of Collink.3D™ 50L solution

Photoinitiator	Wavelength (nm)	Working concentration (% w/v)#	Suggested photoinitiators stock solutions preparation
Irgacure 2959 (2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone)	280-365	0.1	10% w/v in 1:1 Ethanol absolute:DDW
LAP (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate)	365-405	0.05-0.15	Up to 2% w/v in DDW

#Represents the final concentration of the photoinitiator for the hydrogel crosslinking.

NOTE:

- Photoinitiator solutions can be sterilized using a 0.22 µm filter.
- LAP stock solution can also be prepared in a neutral medium, i.e., PBS or culture medium.
- Protect from light.

4.6 Cell embedding

To embed cells in Collink.3D™ 50L-based formulations, first follow instructions in section 4.4 for neutralization, while accounting for the volume of the cells to be added.

For cell culture in Collink.3D™ 50L hydrogels, aseptic conditions are recommended.

1. Add dispersed cells to neutralized Collink.3D™ 50L-based photoinitiator-containing solution.
2. Mix quickly and thoroughly by pipetting (avoid air bubbles).
3. Place under a compatible light source to initiate the curing process.
4. Add cell medium and incubate at 37 °C, 5% CO2.

NOTE: Curing conditions, i.e., type of light source, exposure distance and time, effective exposure intensity and photoinitiator type must be optimized per embedded cell types*.

*Different cell types might present varying sensitivities and responses to photocuring conditions.

4.7 Cell seeding

Cells can be seeded on printed post-cured constructs.

NOTE: It is recommended to use a low adhesion well plate.

1. Incubate the cured constructs at 37 °C, 5% CO2 for at least 3 hours in a sterile PBS/cell medium, during which the media should be replaced several times.
2. Discard the medium and seed the cells on top of the surface of the cured constructs.
3. Incubate the seeded constructs for 15 min at 37 °C, 5% CO2.
4. Add cell medium and place back in the incubator.