Directions For Use

Collink.3D™ 50
Recombinant Human Type I Collagen Methacrylamide
Catalog Number W10199

1. Product Description

Collink.3D™ 50 is a recombinant human type I collagen (rhCollagen) methacrylamide, 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 a curable human collagen with approximately 50% of the primary amines converted into methacrylamides.

The material is supplied in 10 mM HCl, at concentrations ranging from 13 mg/mL to 17 mg/mL. Collink.3D™ 50 can be diluted and formulated with other components such as synthetic acryl/thiol monomers and/or polymers, different types of photoinitiators, dyes and biological additives. Thereafter, formulations can be crosslinked to form hydrogels with diverse properties, for use in a variety of applications.

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

2. Indications for Use

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

Biofabricated constructs composed of Collink.3D™ 50 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 the research, development and manufacture of transplantable tissues, scaffolds and organs with complex architectures, to meet specific properties.

Note that Collink.3D™ 50 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 let the product reach room temperature.
- Avoid heating the pre-cured product above 24 °C.
- Protect the material from light exposure.
- Aseptic practice is recommended.
- Unlike tissue-exctracted collagen, Collink.3D™ 50 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 Dilution

Dilution can be performed with a variety of solvents, including weak acids (low concentration HCl and acetic acid), buffers (phosphate buffer, commercial phosphate bufferer saline (PBS)), cell media, and water. Note that changing the pH and salt concentrations can lead to fibrillogenesis/de-fibrillogenesis, which may affect formulation opacity and viscosity (see section 5).

- Due to material viscosity, it is recommended to dilute by weight (assume a density of 1.0 g/cm^3), 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.
- 20 mM acetic acid may also be used, although it may lead to changes in pH/viscosity.
- For a neutral pH, refer to section 4.3.
- Recommended working concentrations: 3 mg/mL - 10 mg/mL, depending on formulation composition (see Table 1).
- After addition of the diluent, shake, roll or stir the mixture 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 (with the exception of neutralization (see section 4.3) and fibrillogenesis (see section 5)).

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

Table 1: *Dilution of 1 g Collink.3D™ 50 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 case of dilution with PBS, initial neutralization is recommended (see section 4.3).

4.3 Neutralization

Product neutralization to reach physiological conditions is essential when conducting *in-vitro* experiments.

Collink.3D™ 50 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™ 50 solution (up to 2 days storage at 4-8 ^{0}C). It is not recommended to prepare and store a solution of neutralized Collink.3D™ 50 longer than the indicated period.

1. Weigh Collink.3D™ 50 solution into a light-protected tube.
2. Add concentrated NaOH, to a final concentration of 15 mM.
3. Mix gently using short vortex (mild conditions).
4. For a 1 g formulation, add 100 µL 10X PBS to 900 mg Collink.3D™ 50/NaOH solution.
5. Perform short votrtex (mild conditions). The solution will appear opaque (due to fibrillogenesis), and viscosity will increase.
6. Mix at room temperature for 1-2 hours until the solution becomes clear. Avoid the formation of air bubbles. If formed, remove by spin-down.
7. Measure pH (pH test strip may be adequate with small volumes) to ensure neutral pH. Adjust pH by titration.
8. If further dilution is required, first calculate the solution concentration after neutralization (see initial concentration in product label, certificate of analysis). Then, adjust the solution concentration to the desired concentration by diluting with 1X PBS (see section 4.2), 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 2.

Table 2: Collink.3D™ 50 neutralization ratios

Collink.3D™ 50 [g]	NaOH 1 N* [µL]	10X DPBS [µL]	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™ 50 solution is prepared, it is recommended to use lower NaOH stock concentrations, i.e. 0.5 N or 0.1 N.

4.4 Photo-crosslinking

Collink.3D™ 50 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 biofabrication of hydrogels with no additional photocurable ingredients.

NOTE: Collink.3D™ 50 will not form a physicaly crosslinked gel upon heating up to 37°C. Hydrogels can only be achieved by chemical crosslinking. Avoid heating Collink.3D™ 50 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 Collink.3D™ 50 working solution (see dilution instructions in section 4.2), 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 addition of a photoinitiator the Collink.3D™ 50 solution can be stored at 2-8 °C, light protected, for up to 2 days.

Recommended photoinitiators with their respective concentrations and required light source wavelengths are listed in Table 3.

NOTE:

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

Table 3: Recommended photoinitiators for photocrosslinking reaction of Collink.3D™ 50

Photoinitiator	Wavelength (nm)	Working concentration (% w/v)[#]	Suggested photoinitiators stock solutions
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.

4.5 Cell embedding

To embed cells in Collink.3D™ 50-based formulations, first follow instructions in section 4.3 for neutralization, while accounting for the cells volume to be added.

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

1. Add dispersed cells to neutralized Collink.3D™ 50-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% CO_2.

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

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

4.6 Cell seeding

Cells can be seeded on printed post-cured constructs.

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

1. Incubate the cured constructs at 37 °C, 5% CO_2 for at least 3 hours in sterile PBS/cell medium, during which the media should be replaced several times.
2. Discard the medium and seed the cells on the surface of the cured constructs.
3. Incubate the seeded constructs for 15 min at 37 °C, 5% CO_2.
4. Add cell medium and place back in the incubator.

5. Fibrillogenesis

Collink.3D™ 50 fibrillogenesis is strongly affected by the type of buffer used and salts concentration and composition.

Fibrillogenesis of Collink.3D™ 50 can be attained at neutral pH. It is recommended to use sodium phosphate dibasic buffer at a working concentrations range of 16-24 mM.

Recommended concentration range of Collink.3D™ 50 for fibrillogenesis: 9 mg/mL- 12 mg/mL.

A significant decrease in fibrillogenesis efficiency is expected when working with Collink.3D™ 50 concentrations below the indicated range. Higher concentrations yield viscous and non-homogenous solutions.

1. Adjust Collink.3D™ 50 to the target concentration using 10 mM HCl (see section 4.2 for dilution instructions).
2. Add 10X sodium phosphate dibasic buffer (160-240 mM, pH 11.2) at a ratio of 1:9 with respect to Collink.3D™ 50. For example, add 0.1 mL of 10X sodium phosphate dibasic buffer to 0.9 g Collink.3D™ 50 solution.
3. Vortex immediately.
4. Incubate at room temperature, protected from light. Mild shaking is optional. A white, opaque fibril dispersion should be obtained.