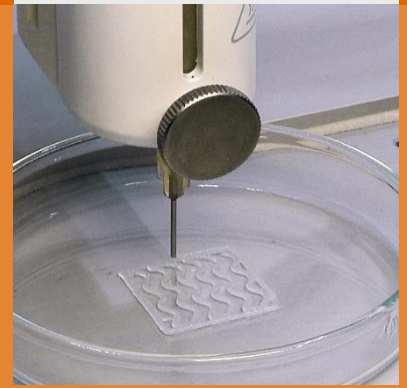


### Fibercoll-Flex-N® preparation and bioprinting

Fibercoll-Flex-N® bioink consists of complex collagen type I fibers from bovine skin. Before 3D bioprinting it is neutralized and loaded with cells and can be printed at temperatures between 4 and 37°C. Scaffold stiffness can be adjusted by dilution of the bioink and no cross-linking is required.

For optimal results please follow this User Guide.



Cell-loaded Fibercoll-Flex-N® bioprint

For technical support contact our team at [sales@bio.viscofan.com](mailto:sales@bio.viscofan.com).

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## GENERAL INTRODUCTION

This User Guide provides instructions to prepare Fibercoll-Flex-N® bioink for 3D bioprinting. Step-by-step, neutralization and cell loading prior to printing are described as well as tips to get a homogenous mixture and avoid air bubbles. The stock suspension can be diluted to concentrations between 2 and 3 wt% collagen. If necessary, the protocol can be adapted to an individual final collagen concentration. Examples for printing conditions are also included.

### Precautions

- Store Fibercoll-Flex-N® bioink at 2 to 8°C. Do not freeze.
- Please use eccentric syringes (provided) for mixing in steps 3 and 4, as homogenous mixing can not be guaranteed with centric syringes.
- We recommend to use a standard 20G needle (provided) for printing - not conical tips.
- Please use appropriate cell culture plastics, media, and reagents as well as aseptic techniques, and ensure adequate conditions for cell growth. Bioprinting may be carried out at temperatures between 4 and 37°C.
- The provided protocol is a proposal and may be varied by the user according to his needs.

### Required material

- 1 syringe containing 3 ml of Fibercoll-Flex-N®\*
- 1 sterile 20G needle\*
- 3 sterile eccentric 10 ml syringes\*
- 1 female-female Luer connector\*
- 1 sterile syringe
- 1 filter (0.22 µm)
- 1 syringe compatible with your bioprinter
- 1 vial with 2 g Tris(hydroxymethyl)aminomethane\*
- HCl (37%)

### INTENDED USE

Fibercoll-Flex-N® is intended for research use only. It is neither intended for human nor animal diagnostic, therapeutic use nor any other clinical use.

\*provided in the kit

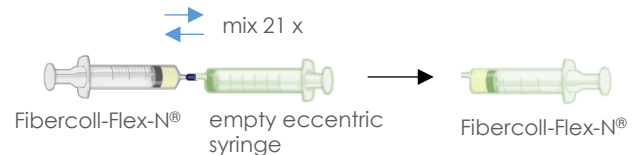
## Fibercoll-Flex-N® preparation and bioprinting

Table 1: Matrix for neutralization and cell loading of Fibercoll-Flex-N® for aimed collagen concentrations from 2 to 3 wt%

Aimed concentration of collagen [wt%]	Ratio of Fibercoll-Flex-N® stock suspension (stock: 5 wt% collagen)	Ratio of 1.5 M Tris-HCl buffer pH 7,3	Ratio of cell culture media containing cells
3	3	1	1
2,5	2,5	1,25	1,25
2	2	1,5	1,5

### STEP 1

A first homogenization step is recommended: Unpack the syringe with Fibercoll-Flex-N® and connect it with an empty eccentric syringe using a junction. Then pass the content from one to the other 21 times, ensuring a homogeneous mixture. Then fill the desired volume of Fibercoll-Flex-N® for printing into the eccentric syringe before you use it in step 3.

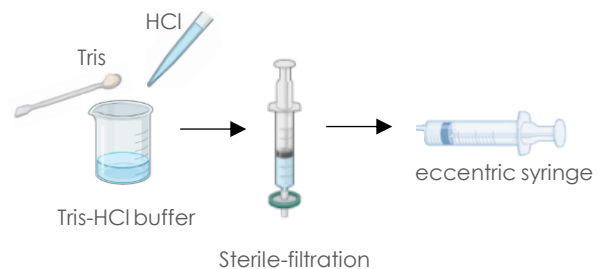


### STEP 2

Prepare the 1.5 M Tris-HCl buffer:

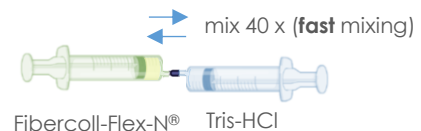
- For 10 ml 1.5 M Tris-HCl buffer, weigh 1,817 g of provided TRIS salt and dilute in ddH<sub>2</sub>O
- adjust it to pH 7,3 with concentrated HCl (37%).
- Sterile-filtrate the solution through a 0,22 µm filter inside a laminar flow chamber and fill it into an eccentric syringe according to the ratio described in table 1.

We recommend to prepare the Tris buffer freshly for use.



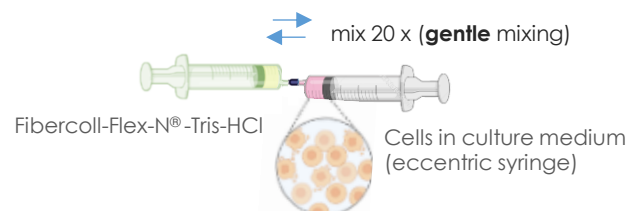
### STEP 3

For neutralization of the Fibercoll-Flex-N® connect both syringes and pass the content from one to the other **40 times quickly** (in less than 30 sec.), ensuring a homogeneous mixture.



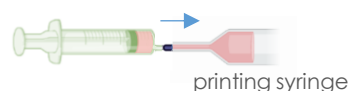
### STEP 4

Fill another eccentric syringe with cells in culture medium according to the ratio described in table 1. Connect it with the syringe containing the neutralized bioink and mix very **gently 20 times**.



### STEP 5

If necessary, transfer the mixture into a syringe suitable for bioprinting.



### Fibercoll-Flex-N® preparation and bioprinting

#### STEP 6

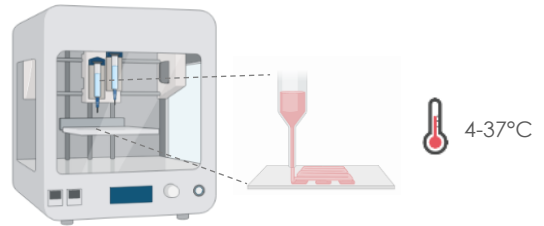
Print the scaffold at the desired temperature between 4 and 37°C.

Recommended conditions for a pneumatic extrusion based bioprinter, using a 20G needle, at 20°C are:

- for 3 wt% collagen: 70-80 kPa 5 mm/s
- for 2 wt% collagen: 50-60 kPa 5 mm/s

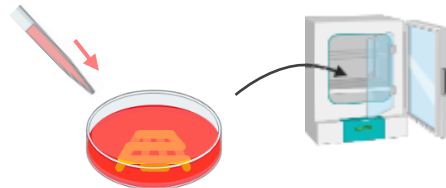
If needed, adjust the conditions changing the pressure and speed of the printer.

Printing at 37°C may slightly reduce the viscosity. For consistent printing results a small pressure reduction may be required.



#### STEP 7

Add cell culture media until the scaffold is completely submerged and incubate at suitable conditions.



*Most tool-symbols were derived from BioRender.*

PROBLEM	ANSWER
1. In case my bioink has <b>air bubbles</b> , are they going to interfere with printability or cell culture?	<ul style="list-style-type: none"> <li>• Small bubbles will not interfere in printing. A centrifugation step before mixing with the cells can be performed to remove most air bubbles.</li> <li>• The homogenization step (step 1) will reduce the size and number of air bubbles to facilitate the printing process.</li> </ul>
2. I do not want to use the whole syringe at once. Can I use only a part of it?	After homogenization in step 1, transfer the desired amount of Fibercoll-Flex-N® to a sterile syringe and continue the protocol. To neutralize it, follow the same ratio Fibercoll-Flex-N® : Tris-HCl buffer : culture media described in "Table 1"
3. I can't screw together both syringes with the connector. How can I avoid spilling of the bioink?	Eccentric syringes to date can't be connected by screwing. However, homogeneous mixing at steps 3 and 4 can not be guaranteed when using central (Luer-lock) syringes. To avoid spilling of bioink, use the connector provided and carefully exert pressure from both ends of the syringes when mixing.
4. <b>How fast</b> should I do the <b>mixing</b> steps?	There are 3 mixing steps during the process with different demands: <ol style="list-style-type: none"> <li>1. Homogenization (step 1): Pass Fibercoll-Flex-N® to an empty syringe and back 20 times in about 1 minute.</li> <li>2. Neutralization (step 3): Mix Fibercoll-Flex-N® with Tris-HCl buffer 40 times very quickly in less than 30 seconds.</li> <li>3. Mixing with cells (step 4): Mix the neutralized bioink with cells in medium 20 times very gently to minimize shear stress.</li> </ol>
5. The <b>extrusion</b> of bioink is <b>not continuous or not homogenous</b> .	<ul style="list-style-type: none"> <li>• Make sure that you are using the needle (provided in the kit) or similar for printing. Do not use conical tips.</li> <li>• Make sure that the printing pressure is high enough to to extrude the chosen collagen concentration of the Fibercoll-Flex-N® (note recommended settings in step 6).</li> <li>• Make sure to use the eccentric syringes (provided) or similar for the mixing in steps 3 and 4. If centric syringes are used, homogeneity cannot be ensured.</li> <li>• To avoid interference of air bubbles please consider problem &amp; answer 1.</li> </ul>

*All data and recommendations correspond to the present state of our knowledge; they are published without engagement. We reserve the right to make alterations and additions in line with technical developments without prior notice. The customer is obliged to check whether our products meet the technical requirements. Please contact us for questions or support.*



**Fibercoll-Flex-N®**

VWR catalog number: 76628-718

**Contact us for support**

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