# USER GUIDE

## 3D-PRINTING WITH FIBERCOLL-FLEX-A®

**Fibercoll-Flex-A® preparation, adjustment of collagen concentration, printing and neutralization**

Fibercoll-Flex-A® is an acidic bioink consisting of complex collagen type I fibers. After 3D printing and neutralization, it forms highly stable collagen scaffolds without the need for chemical crosslinking, creating customizable matrices for cell seeding. Scaffold stiffness can be adjusted by diluting the bioink. Before cell seeding, the printed scaffold must be neutralized.

For optimal results please follow this User Guide.

**For technical support contact our team at sales@bio.viscofan.com.**

*Printed Fibercoll-Flex-A® scaffold*



## GENERAL INTRODUCTION

This guide explains how to prepare Fibercoll-Flex-A® bioink for 3D printing to ensure a homogenous mixture while avoiding air bubbles. It includes step-by-step instructions for diluting the stock ink to easily create working inks with 2, 3, 4 or 5 wt% collagen. The protocol can also be adapted for other desired final collagen concentrations.
This guides includes examples for printing conditions and a neutralization protocol for the printed scaffold before cell seeding.

### Precautions

- Store the Fibercoll-Flex-A® bioink at 2 to 8°C. Do not freeze.
- Bioprinting may be carried out at temperatures between 4 and 37°C.
- We recommend using a standard 20G needle (provided) for printing instead of conical tips.
- Please use appropriate cell culture plastics, media, and reagents as well as aseptic techniques, and ensure appropriate conditions for cell growth.
- The provided protocol represents a proposal and may be varied by the users according to their needs.

### Required material

- Syringe containing 3 ml of Fibercoll-Flex-A®*
- Sterile 20G needle*
- Sterile syringes (luer-lock or other)
- Sterile connector
- Sterile syringe compatible with your bioprinter
- Sterile NaOH, 0.05M
- Sterile PBS
- Sterile cell culture medium
- Optionally: centrifuge
- Optionally: Sterile $dH_2O$

**INTENDED USE**

Fibercoll-Flex-A® 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*



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## Fibercoll-Flex-A® working ink preparation, adjustment of collagen concentration, printing and neutralization

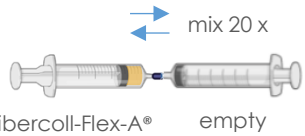
Table 1: Dilution matrix for collagen concentrations of 2 to 5 wt%

| Aimed concentration of collagen [wt%] | Fraction of Fibercoll-Flex-A® stock suspension (stock: 5 wt% collagen) | Fraction of sterile $dH_2O$ |
|---|---|---|
| 5 | 1 | 0 |
| 4 | 4 | 1 |
| 3 | 3 | 2 |
| 2 | 2 | 3 |

**STEP 1**

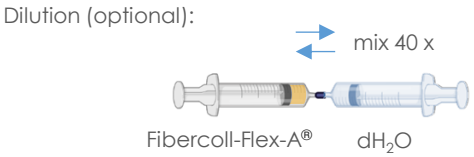
We recommend a homogenization step: For this, unpack the syringe containing the Fibercoll-Flex-A® stock ink and connect it using a connector to a sterile, empty syringe, taking care to minimize air entrapment. Transfer the complete bioink back and forth between the two syringes 20 times to achieve a homogeneous mixture.

Fill the desired volume of Fibercoll-Flex-A® for printing or dilution into one syringe. If no dilution to reduce the collagen concentration is desired, proceed to step 3.




**STEP 2**

If dilution is desired, connect the syringe containing Fibercoll-Flex-A® to a syringe filled with sterile $dH_2O$ at the ratio described in table 1 and transfer the entire content back and forth between the syringes 40 times to ensure a homogeneous mixture.

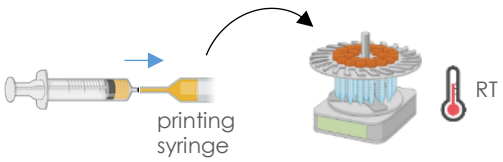



**STEP 3**

If necessary, transfer the bioink to a syringe compatible with your bioprinter.

If reduction of the number of small air bubbles is desired, we recommend to centrifuge the ink. For more information, see the trouble shooting section, issues #1 and #2.

The Fibercoll-Flex-A® working ink is now ready for printing.

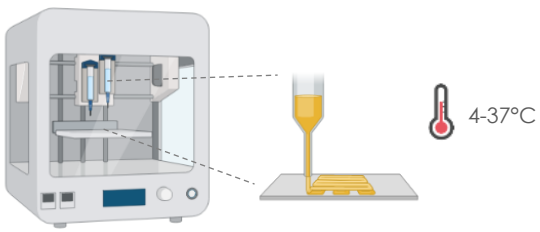



**STEP 4**

Print the scaffold at the desired temperature between 4 and 37°C. We recommend the following conditions as a basis for printing with a pneumatic extrusion based bioprinter, using a 20G needle, at 20°C:

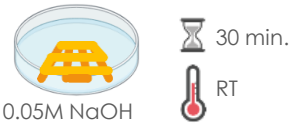
- for 5 wt% collagen: 300 kPa, 5 mm/s
- for 3 wt% collagen: 110-150 kPa, 5 mm/s

If needed, adjust the conditions by changing the pressure and printhead or table speed of the printer.




**STEP 5**

After printing, neutralize the scaffold by fully covering it with sterile 0.05M NaOH for 30 min. at room temperature.



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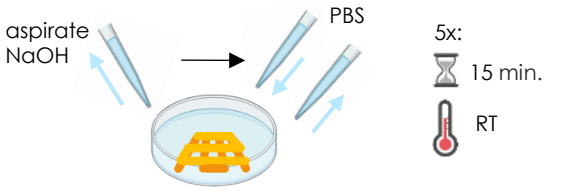
## Fibercoll-Flex-A® working ink preparation, adjustment of collagen concentration, printing and neutralization

**STEP 6**

Discard the NaOH and add PBS until the scaffold is completely covered. Incubate for 15 min. at room temperature.

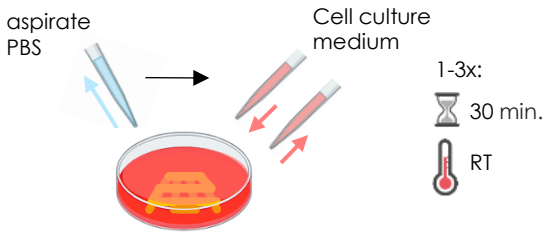
Repeat the washing step four more times.




**STEP 7**

Discard the PBS and cover the scaffold completely with cell culture medium for 30 min. at room temperature.

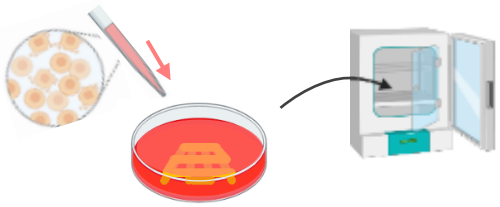
The scaffold should have turned rose by now (in case the medium contains phenol red). If the scaffold is fuchsia pink or white, carry out two more 30-min.-washes before cell seeding.




**STEP 8**

The scaffold is now ready for cell seeding.

After seeding, we recommend covering the scaffold completely with cell culture medium before transferring it to suitable culture conditions.



*Most tool-symbols are derived from BioRender.*

| ISSUE | TIPS |
|---|---|
| 1. Will air bubbles in my working ink interfere with printability? | • Small air bubbles will not interfere with printing. The homogenization step (step 1) will reduce the size of potential air bubbles.<br>• If reduction of the amount of air bubbles is desired, please see issue 2. |
| 2. How can I reduce small air bubbles in the working ink? | • When connecting syringes for homogenization or dilution in steps 1, 2 and 3, avoid introducing air as much as possible.<br>• To reduce the number of small air bubbles before step 4, we recommend allowing the working ink to rest and equilibrate to room temperature (RT) in an upright position for e.g. 2 h followed by centrifugation at RT. Please note: higher collagen concentrations require increased centrifugation speed and time. Recommended values are 3200 to 9000 x g for 10-50 min.<br>• Efficiency of air bubble removal can be further improved by allowing the working ink to relax at 4-8°C for several hours after step 2, before equilibrating to RT and centrifuging as described above.<br>• Alternatively, small air bubbles in your printed product after step 4 can be removed by incubating it overnight in a humid chamber before proceeding to neutralization in step 5. Efficiency may vary with collagen concentration, filament and product thickness. |
| 3. Extrusion is not continuous or not homogenous. | • We recommend using the type of needles provided in the kit for printing and advise against conical tips in order to achieve homogenous printing.<br>• Ensure that the printing pressure is adjusted to the chosen collagen concentration and nozzle width. In addition, the print head or table speed and the ink flow rate must be synchronized to ensure consistent printing performance. For this, use our recommendations at step 4 as a basis. If necessary, consider using an external pump to print at higher pressures.<br>• To achieve consistent extrusion, select a proper piston to push down the ink in the syringe during the printing process that fits your printer's pressure-executing technology as well as the ink viscosity. For example, use tightly fitting pistons for mechanical piston-based pressure systems and pistons with a looser fit for pneumatic systems. Submerge the lower part of the piston in the ink and ensure that the ink is sitting compactly at the bottom of the printing syringe.<br>• Ensure that potential air bubbles are small in size (view also issue 1). |



**Fibercoll-Flex-A®**

VWR catalog number: 76628-720

**Contact us for support**

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