# USER GUIDE

**Soluble Collagen**
order-number: 500060635

## Preparation of hydrogels for 3D cell culture with soluble collagen

Soluble collagen can be polymerized to a porous three-dimensional matrix for 3D cell culture. Cells may be embedded inside the hydrogel (protocol A) or seeded on top (protocol B). Please refer to your preferred protocol below.
The protocols are calculated for soft hydrogels with a collagen end concentration of 1,35 mg/ml (orange tables) or stiffer hydrogels with 3 mg/ml (blue tables) collagen end concentration, respectively. If necessary, they have to be adapted to the individual collagen concentration desired. However, the ratio of soluble collagen to NaOH should stay constant in order to result in a hydrogel with neutral pH. Volumes are calculated for soluble collagen with the **lot-number 20200421-0001**.
Liquids should be kept on ice until hydrogel polymerization is intended. Polymerization is induced by rising the pH and temperature.
When working with soluble collagen, please use appropriate cell culture plastics, media, and reagents as well as aseptic techniques, and ensure adequate conditions for cell growth.
For technical support contact our team at sales@bio.viscofan.com.

*Collagen hydrogel for 3D cell culture*



**Materials needed:**

- Soluble collagen from Viscofan Bioengineering
- Pipettes
- Multiwell plate
- Cell culture medium (e.g. DMEM/Glutamin + 10% FCS)
- Sterile NaOH, 1M
- Optionally: analytical pH-paper, range 0-14

### Protocol A: Preparation of collagen hydrogels with cells embedded inside the hydrogel (Soluble collagen Lot# 20200421-0001)

| Table 1 | | 1a | 2a | 3a | 4a | 5a | 6a |
|---|---|---|---|---|---|---|---|
| **Cells embedded in the hydrogel** | **well plate format (volumes for 4 wells*)** | **Soluble collagen [µl]** | **1 M NaOH [µl]** | **Cell culture medium [µl]** | **Cell suspension (4-fold of the intended cell end concentration) [µl]** | **End volume of mixture 1a-4a [µl]** | **Transfer volume of mixture 5a per well* [µl]** |
| For **1,35 mg/ml** collagen end concentration | 4 x 48-well | 650 | 58 | 1 000 | 570 | 2 278 | 500 |
| | 4 x 24-well | 1 300 | 115 | 2 000 | 1 140 | 4 555 | 1 000 |
| | 4 x 12-well | 2 600 | 230 | 4 000 | 2 277 | 9 107 | 2 000 |
| | 4 x 6-well | 5 200 | 460 | 8 000 | 4 555 | 18 215 | 4 000 |
| For **3 mg/ml** collagen end concentration** | 4 x 48-well | 1 444 | 125 | 144 | 570 | 2 283 | 500 |
| | 4 x 24-well | 2 900 | 250 | 287 | 1 140 | 4 577 | 1 000 |
| | 4 x 12-well | 5 780 | 500 | 574 | 2 277 | 9 131 | 2 000 |

*Suggested transfer volumes will result in hydrogels of appr. 1 cm thickness. To produce thinner hydrogels, transfer volumes may be adjusted.

**Hydrogels with high collagen concentration will polymerize faster. To avoid uneven polymerization, be careful to work fast during the steps 2-5 in protocol A.

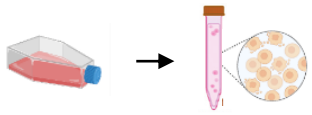


Viscofan BIOENGINEERING

## Protocol A: Preparation of collagen hydrogels with cells embedded inside the hydrogel (Soluble collagen Lot# 20200421-0001)

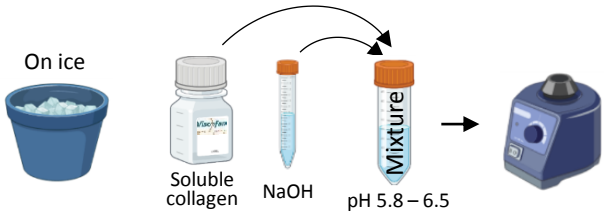
**STEP 1**

Prepare a cell suspension in cell culture medium to be used in step 4, with a 4-fold higher cell concentration than the desired cell end concentration in the hydrogel (e.g., if an end concentration of $1{,}5x10^5$ cells per ml in the hydrogel is intended, prepare a cell suspension of $6x10^5$ cells/ml)



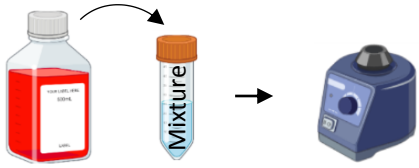

**STEP 2**

Prepare on ice in a second vial: according to the volumens in table 1, first mix the soluble collagen (column 1a) briefly with NaOH (column 2a) by vortexing.




**STEP 3**

Quickly mix thoroughly with cell culture medium (column 3a) by vortexing.

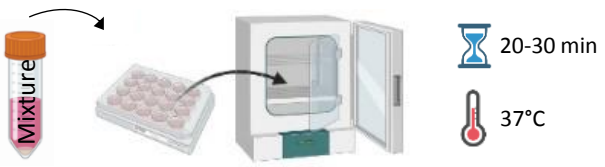



**STEP 4**

Quickly mix briefly with the cell suspension (column 4a) by vortexing.
If required, pipette a few drops on pH-paper to check whether the pH is neutral.


**STEP 5**

Quickly pipette the mixture into the well plate avoiding bubbles (column 6a). Let the hydrogel polymerize completely at 37°C for approximately 20 - 30 min and don't move the wellplate during this time. Continue with step 6 not later than 45 min after step 5.




**STEP 6**

Optionally, wash the hydrogels gently with medium once, then cover the hydrogels with medium and incubate at the usual culture conditions (e.g. at 37°C and 5% $CO_2$).

## Protocol B: Preparation of collagen hydrogels with cells seeded on top (soluble collagen Lot# 20200421-0001)

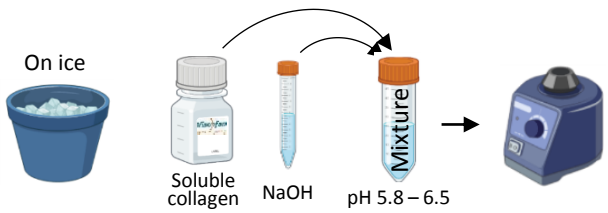
| Table 2 | | 1b | 2b | 3b | 4b | 5b |
|---|---|---|---|---|---|---|
| Cells seeded on top | well plate format (volumes for 4 wells*) | Soluble collagen [µl] | 1 M NaOH [µl] | Cell culture medium [µl] | End volume of mixture 1b-3b [µl] | Transfer volume of mixture 4b* per well [µl] |
| For **1,35 mg/ml** collagen end concentration | 4 x 48-well | 650 | 58 | 1 570 | 2 277.5 | 500 |
| | 4 x 24-well | 1 300 | 115 | 3 140 | 4 555 | 1 000 |
| | 4 x 12-well | 2 600 | 230 | 6 280 | 9 110 | 2 000 |
| | 4 x 6-well | 5 200 | 460 | 12 560 | 18 219 | 4 000 |
| For **3 mg/ml** collagen end concentration** | 4 x 48-well | 1 444 | 125 | 714 | 2 283 | 500 |
| | 4 x 24-well | 2 900 | 250 | 1 427 | 4 577 | 1 000 |
| | 4 x 12-well | 5 780 | 500 | 2 851 | 9 131 | 2 000 |

*Suggested transfer volumes will result in hydrogels of appr. 1 cm thickness. To produce thinner hydrogels, transfer volumes may be adjusted.

**Hydrogels with high collagen concentration will polymerize faster. To avoid uneven polymerization, be careful to work fast during the steps 1-3 in protocol B.

**STEP 1**

Prepare on ice: according to the volumens in table 2, first mix the soluble collagen (column 1b) with NaOH (column 2b) by vortexing.

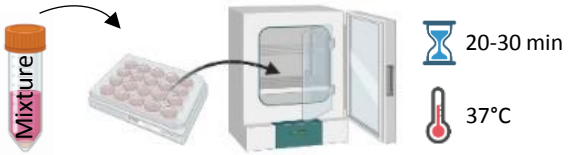



**STEP 2**

Quickly mix thoroughly with cell culture medium (column 3b) by vortexing.

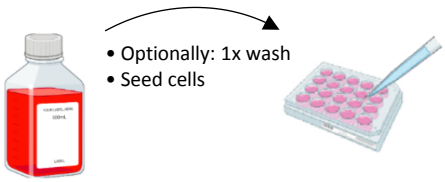

**STEP 3**

Quickly pipette the mixture into the wellplate avoiding bubbles (column 5b). Let the hydrogels polymerize completely at 37°C for approximately 20 - 30 min and don't move the well plate during this time. Continue with step 4 not later than 45 min after step 3.




**STEP 4**

Optionally, wash the hydrogels gently with medium once.
The hydrogels are now ready for cell seeding.
Cover the hydrogels with medium until cell seeding to avoid drying. Also cover the hydrogels with medium after cell seeding.




Tool-symbols are derived from BioRender

Trouble shooting:

1. If the hydrogel fails to polymerize, please check whether the pH is neutral. After step 2 in protocol A and step 1 in protocol B the pH should lie between 5.8 and 6.5 in order to result in neutral pH in the finished collagen hydrogel. If in doubt, we recommend to do a pre-test using a pH-meter and to adjust the amount of NaOH, if necessary.

   Don‘t move the well plate during the hydrogel polymerization step (step 5 in protocol A and step 3 in protocol B, respectively).

2. If cells die or fail to grow, please check whether the pH is neutral. Please check whether the hydrogel density or the seeding density is suitable for your kind of cells and whether enough cell culture medium has been added to the hydrogel.

3. The protocols are designed to result in neutral pH in the finished mixture. Especially with high collagen concentration, hydrogels might appear yellowish shortly after polymerization but change to red within 1-2 hours. If in doubt whether neutral pH has been achieved, please check the pH of the final mixture using pH paper or do a pre-test using a pH-meter.

   Please ensure that the lot of your soluble collagen fits the lot, the protocol is calculated for.

4. Due to the high collagen concentration, our soluble collagen has a high viscosity. To avoid pipetting errors, please pipette slowly. Additionally, only dip the end of the pipette tip into the collagen solution and let collagen solution that sticks to the outside of the pipette tip flow back before transferring to the mixture tube.

**Intended use**

Soluble collagen is intended for research use only. It is neither intended for human nor animal diagnostic, therapeutic use or any other clinical use.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order-number: 500060635



**Contact us**
sales@bio.viscofan.com
+49 (06201) 86-358


Use collagen scaffolds to improve performance of your 2D & 3D cultures!

**Viscofan BioEngineering**
A Business Unit of Naturin Viscofan GmbH
Badeniastraße 13 • 69469 Weinheim, Germany
Phone +49 (0)6201 86-358 • Fax +49 (0)6201 86-226
sales@bio.viscofan.com • www.viscofan-bioengineering.com



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