USER GUIDE

GENERATION OF HYDROGELS WITH BOVINE SOLUBLE COLLAGEN

Preparation of hydrogels for 3D cell culture with Bovine Soluble Collagen

Bovine Soluble Collagen can be polymerized to a 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.

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

Collagen hydrogel for 3D cell culture

GENERAL INTRODUCTION

Viscofan BioEngineering's Bovine Soluble Collagen has a pH of ≅3.5 and a collagen concentration of ≅5 mg/ml depending on the lot*. Optimized for a certain lot, the following protocols are calculated to generate hydrogels with a final collagen concentration of 1.35 mg/ml and neutral pH. Volumes are calculated for Bovine Soluble Collagen with the lot number 20220207-0002. If necessary, they can be adapted to the individual collagen end concentration desired (please note the trouble shooting section).

**The exact collagen concentration and pH-value for each specific lot is provided in the Certificate of Analysis supplied with the purchase of each product.*

Precautions

- 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.
- Due to the high collagen concentration, our Bovine Soluble Collagen has a high viscosity. To avoid pipetting errors, please pipette slowly to allow complete pouring in and out, respectively. Additionally, only dip the end of the pipette tip into the Bovine Soluble Collagen and discard any collagen solution that sticks to the outside of the pipette tip before transferring the correct volume to the mixture tube.

Required material

- Bovine Soluble Collagen, lot number 20220207-002
- Pipettes
- Multi well plate
- Cell culture medium (e.g. DMEM/Glutamin + 10% FCS)
- Sterile NaOH, 1M
- Optionally: analytical pH-paper, range 0-14

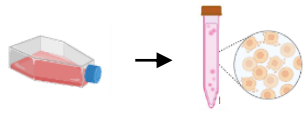
INTENDED USE

Bovine Soluble Collagen is intended for research use only. It is neither intended for human nor animal diagnostic, therapeutic use nor any other clinical use.

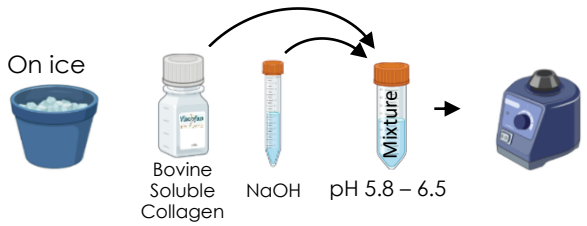
Table 1		1a	2a	3a	4a	5a	6a
For cells embedded in the hydrogel	**Well plate format (volumes for 4 wells*)**	**Bovine Soluble Collagen [µl]**	**1 M NaOH [µl]**	**Cell culture medium [µl]**	**Cell suspension [µl] (4-fold of the intended cell end concentration)**	**End volume of mixture 1a-4a [µl]**	**Transfer volume of mixture 5a per well* [µl]**
For **1.35 mg/ml** collagen end concentration	4 x 48-well	769	60	879	570	2 278	500
	4 x 24-well	1 538	118	1 760	1 139	4 555	1 000
	4 x 12-well	3 075	236	3 522	2 277	9 110	2 000

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

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 final cell concentration in the hydrogel (e.g., for a final concentration in the hydrogel of $1.5x10^5$ cells/ml, prepare a cell suspension of $6x10^5$ cells/ml)



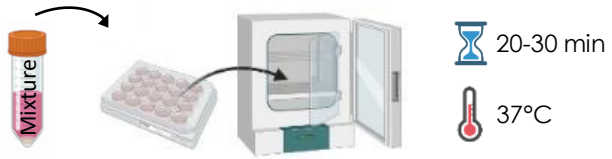
STEP 2 Prepare on ice in a second vial: according to the volumes in table 1, first mix the Bovine Soluble Collagen (column 1a) briefly with NaOH (column 2a) by vortexing. The pH of the mixture should lie between 5.8 and 6.5.



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 well plate during this time.



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).

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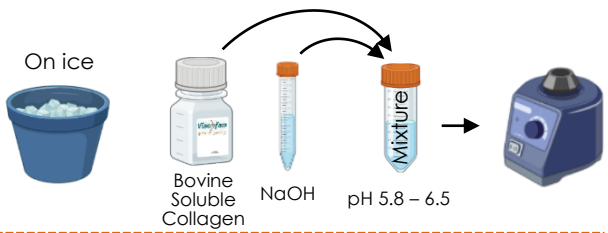
Table 2		1b	2b	3b	4b	5b
For cells seeded on top	Well plate format (volumes for 4 wells*)	Bovine Soluble Collagen [µl]	1 M NaOH [µl]	Cell culture medium [µl]	End volume of mixture 1b-3b [µl]	Transfer volume of mixture 4b* per well [µl]
For **1.35 mg/ml** collagen end concentration	4 x 48-well	769	60	1 449	2 278	500
	4 x 24-well	1 538	118	2 899	4 555	1 000
	4 x 12-well	3 075	236	5 799	9 110	2 000

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

STEP 1

Prepare on ice: according to the volumes in table 2, first mix the Bovine Soluble Collagen (column 1b) with NaOH (column 2b) by vortexing. The pH of the mixture should lie between 5.8 and 6.5.

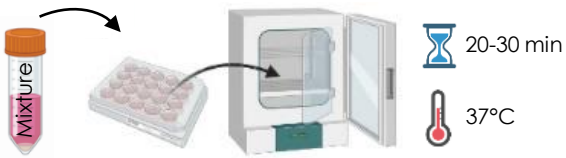


STEP 2

Quickly mix thoroughly with cell culture medium (column 3b) by vortexing.
If required, pipette a few drops on pH-paper to check whether the pH is neutral.

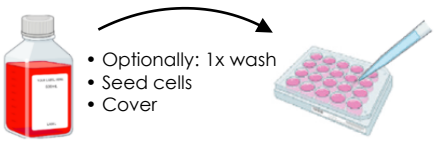
STEP 3

Quickly pipette the mixture into the well plate avoiding bubbles (column 5b). Let the hydrogels polymerize completely at 37°C for approximately 20 - 30 min and do not move the well plate during this time.



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 generated with BioRender

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PROBLEM	SOLUTION
1. Hydrogel fails to polymerize	• Please check whether the pH of the final mixture is neutral and adjust if necessary (see 5.). • Do not move the well plate during the hydrogel polymerization step (step 5 in protocol A and step 3 in protocol B, respectively).
2. Hydrogel does not polymerize homogenously	• Especially when aiming at high collagen concentrations, be sure to work fast after addition of NaOH (steps 2-5 in protocol A and steps 1-3 in protocol B) • Keep liquids on ice until hydrogel polymerization is intended (when rising pH and temperature).
3. Hydrogel appears yellowish instead of red	• Especially with high collagen concentrations, 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 whether the pH of the final mixture is neutral and adjust, if necessary (see 5.).
4. Cells die or fail to grow	• Please check whether the pH of the final mixture is neutral and adjust if necessary (see 5.) • Please check whether the hydrogel density and the cell seeding density is suitable for your kind of cells. • Please check whether enough cell culture medium has been added to or on top of the hydrogel to feed the cells. • Use a gentler mixing method in protocol A step 4.
5. Ensuring, checking and adjusting pH of the hydrogel	• The protocols are designed to generate hydrogels with neutral pH. Please ensure that the lot of your Bovine Soluble Collagen corresponds to the lot, the protocol is calculated for. • If adjusting the protocol for a different collagen end concentration is intended or if generally in doubt, whether neutral pH has been achieved, we recommend performing a pre-test using a pH-meter and adjusting the amount of NaOH, if necessary. As a guideline: after addition of NaOH (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 for the finished collagen hydrogel. • Alternatively, as a quick check, pipette a few drops of the final mixture on pH paper before gelation starts. • Pipette slowly to avoid pipetting errors due to the slow flowing properties of the viscous Bovine Soluble Collagen. Avoid transfer of collagen solution that sticks to the outside of the pipette tip.

Bovine Soluble Collagen
Order-number: 500060635

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

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