

Preparation of *3-D Life* Fast Gelling Hydrogels

1. Introductory Notes

- The *3-D Life* Hydrogel System is a complete set of reagents for the design of extracellular microenvironments of three-dimensional cell cultures and related applications. Ease of use and complete control of biomolecular modifications and gel stiffness allow a great variety of cell culture applications.
- The polymers Mal-Dextran and Mal-PVA are used to generate hydrogels at a very fast gelation rate. Incubation times for the attachment of adhesion peptides takes only 5 minutes and gel formation concludes instantaneously, within seconds or a few minutes depending on the gel composition and pH applied.
- Fast gelling hydrogels are used when the formation of hydrogels has to be rapid. For example, when cells need to obtain appropriate medium within a few minutes, or when the application requires a very rapid gelation, for example for bioprinting. To allow enough time for manually placing the gel, gel formation is performed at pH 5.5. For instantaneous gel formation CB pH 7.2 is used.
- The *3-D Life* Hydrogel technology and its applications are described in detail in the *3-D Life* Hydrogels User Guide which can be downloaded at cellendes.com. For first time users it is recommended to read the User Guide carefully before setting up gels.

2. Protocol

The following protocol describes the preparation of *3-D Life* Hydrogels for 3-D cell culture with and without modification with the cell adhesion peptide *3-D Life* RGD Peptide.

Reagents and materials

***3-D Life* products:**

3-D Life Dextran-CD Hydrogel FG (Catalog Number FG91-1)

or *3-D Life* Dextran-PEG Hydrogel FG (Catalog Number FG90-1)

or *3-D Life* PVA-CD Hydrogel FG (Catalog Number FG81-1)

or *3-D Life* PVA-PEG Hydrogel FG (Catalog Number FG80-1)

Optional: *3-D Life* RGD Peptide (Catalog Number 09-P-001)

Related products: *3-D Life* Dextranase (Catalog Number D10-1), *3-D Life* Thioglycerol (Catalog Number T10-3)

Reagents and materials not included in the *3-D Life* product:

Cell culture medium, cell culture plate, reaction tubes, pipet tips, micropipets, serological pipets, cell suspension

Preparations

Hydrogel reagents:

- If hydrogel reagents are provided in lyophilized form, dissolve the lyophilisates according to the instructions in the accompanying Product Data Sheets.
- If hydrogel reagents are frozen, thaw all reagents at room temperature. Make sure that salts in the 10x CB are completely dissolved. Do not put 10x CB on ice, this may cause the salts to crystallize.

Note: Do not expose *3-D Life* thiol-containing reagents (RGD Peptide, CD-Link, PEG-Link) to air and room temperature longer than necessary to avoid oxidation of the thiol-groups. Close cap after each use.

Cell suspension:

Prepare a stock cell suspension or any other biological sample of your choice in culture medium, PBS or in any other physiological solution. When preparing this sample, consider that the volume of this sample will be only 1/5 of the final gel volume. Accordingly, the cell concentration in the gel will be only 1/5 of the stock cell suspension.

Experimental procedure

The following protocol describes the preparation of a soft hydrogel (crosslinking strength of 2 mmol/L) with the option of modification with 0.5 mmol/L RGD Peptide. The volumes of gel reagents required for a 30 μ l gel are listed in Table 1.

Note: It is advisable to calculate reagents with some excess volume (e.g. one additional gel volume) to avoid shortage of pre-gel solution due to pipetting inaccuracies.

If not indicated otherwise all steps below are performed in a sterile hood at room temperature:

1. Combine Water, 10x CB (pH 5.5) and Mal-Polymer of your choice (Mal-Dextran or Mal-PVA) in a reaction tube. Mix well.
2. If RGD Peptide is used (otherwise continue with step 3.):
Add the RGD Peptide and mix immediately to ensure homogenous modification of the Mal-Polymer with the peptide. Incubate sample for 5 min to allow for the attachment of the RGD Peptide to the maleimide groups of the Mal-Polymer.
3. Place 3.0 μ l of crosslinker (CD-Link or PEG-Link) on the surface of a culture dish.
4. Add the cell suspension to the reaction tube containing the peptide-polymer conjugate to complete Reagent mix A.
5. In a pipet tip, transfer 27 μ l of Reagent mix A to the 3.0 μ l crosslinker (PEG-Link or CD-Link) on the dish and quickly mix both by pipetting up and down three times. Avoid the formation of air bubbles. Leave the mix on the surface of the culture dish. Wait for approximately 3 minutes to let the gel form.

Note: Gel formation starts after a few seconds of mixing. Complete the mixing step as fast as possible to avoid gel formation in the pipet tip. Make sure that the gel has completely formed before adding culture medium in step 6. Optional: Test gel formation by careful inspection with a pipet tip. The tip should not pull out threads of gel when touching and retracting from the gel surface.

6. Once the gel has formed, add cell culture medium until the gel is covered.
7. Place culture dish in the incubator for cultivation of cells.
8. Renew the medium after 1 hour.
9. Change the medium as needed during cultivation of cells.

Table 1: Reagent volumes for a 30 μ l gel using Mal-Dextran or Mal-PVA polymer to be crosslinked with 2 mmol/L SH groups of the crosslinker CD-Link or PEG-Link (2 mmol/L crosslinking strength) with the option of modification with 0.5 mmol/L RGD Peptide.

Reagents	Final concentrations in the gel	Volumes for 30 μ l gels (μ l)	
		w/o peptide	with peptide
Water	n.a.	16.6	15.3
10x CB, pH 5.5	n.a.	2.4	2.4
Mal-Dextran or Mal-PVA (30 mmol/L maleimide groups)	2.0 mmol/L (w/o peptide) or 2.5 mmol/L (with peptide) maleimide groups	2.0	2.5
RGD-Peptide (20 mmol/L SH groups)	0.5 mmol/L SH groups	-	0.8
Cell suspension	user's choice	6.0	6.0
PEG-Link or CD-Link (20 mmol/L SH groups)	2 mmol/L SH groups	3.0	3.0
Total		30.0	30.0

Variations of gel preparation

Reagent volumes for gel variations described below can be easily calculated using the online calculation tool on cellendes.com.

Preparation of small gel volumes:

If small volumes of gels are prepared (less than 100 μ l) only very small volumes of the RGD Peptide stock solution are required. To avoid the pipetting of such small volumes, it is recommended to reduce the concentration of the RGD Peptide stock solution (e.g. 3 mmol/L) by dilution with water to increase the volume to be pipetted. In this case the volume of the component "Water" has to be adjusted accordingly.

Preparation of multiple gels of same composition:

For the preparation of multiple gels of the same composition Reagent mix A consisting of Water, 10 x CB pH 5.5, Mal-Polymer (Mal-Dextran or Mal-PVA), RGD Peptide and cell suspension can be prepared for all gels in one volume. For final gel formation aliquots of the Reagent mix A are combined with appropriate volumes of crosslinker (CD-Link or PEG-Link). It is recommended to resuspend cells in the Reagent mix A before pipetting each aliquot to ensure an equal number of cells in each gel.

Preparation of gels of larger volumes:

If larger volumes of gel are prepared, reagents and cell suspension are prepared in multiples of the indicated volumes or adapted proportionally in volume.

Preparation of gels of different stiffness:

Gels of higher stiffness than the gels described in Table 1 can be made by increasing the concentrations of the Mal-Polymer (Mal-Dextran or Mal-PVA) and crosslinker (CD-Link or PEG-Link). For calculating reagent volumes, please consult the User Guide or the online calculator.

Preparation of gels with different concentrations of RGD Peptide:

If gels of different concentrations of adhesion peptide are to be prepared, please consult the User Guide or our online calculator for calculating volumes of reagents.

Preparation of plain gels (without cells) or embedding other specimens:

If no cells are included in the gel, e.g. for encapsulation of tissues or preparation of plain gels, replace the volume of cell suspension with PBS or other physiologically compatible solution of your choice. Alternatively, use the online calculator and keep the component cell suspension blank or enter "0".

RGD Peptide replacements for control experiments:

Instead of the RGD-Peptide, Thioglycerol (*3-D Life* Thioglycerol, Catalog Number T10-3) can be added to the gel. In this case the gel does not provide cell attachment sites and can be used as a control for RGD Peptide-modified gels. Cellendes also offers a scrambled version of RGD Peptide for control experiments (*3-D Life* Scrambled Peptide, Catalog Number 09-P-003).

Dissolving Dextran Hydrogels with Dextranase

Dextran Hydrogels containing live or chemically fixed cells can be dissolved by adding dextranase to the culture medium or buffer. For example, a 30 μ l gel can be dissolved with 300 μ l of a 1:20 dilution of dextranase in medium incubated for 30-60 minutes at 37°C. Gels can be dissolved faster, if they are cut in pieces.

After dissolution of the gel, centrifuge the cell suspension and resuspend the pelleted cells in fresh medium or physiological buffer as required. Repeat this washing procedure once or twice to more effectively remove remains of dextranase and dissolved gel components. The removal of dextranase is important when cells are being embedded again in dextran hydrogels to continue culture. If dextranase is not removed completely, it can destabilize the newly set up hydrogel.