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Lab & Production Materials

Specification Sheet

QUICK PROTOCOL

TrueGel3D™ hydrogel CD cell-degradable crosslinker and RGD peptide (TRUE1) preparation

Introduction

TrueGel3D™ is a chemically defined hydrogel formed by mixing polymers with crosslinkers. This hydrogel allows encapsulating cells to put them in a 3 dimensions environment to better mimic native tissues environment.

The TrueGel3D™ hydrogel CD cell-degradable crosslinker and RGD peptide offer a predefined combination of reagents that allow cell growth in a 3D hydrogel with standard characteristics:

- Appropriate density to provide a suitable environment for a variety of cells
- A pre-defined concentration of RGD motif for cell adhesion
- A CD (cell-degradable) crosslinker to allow cell spreading and migration inside the hydrogel
- Cells can be recovered after 3D experiments for downstream analysis by using the TrueGel3D™ enzymatic cell recovery solution

TrueGel3D™ hydrogel CD cell-degradable crosslinker and RGD peptide is recommended as a starting point for 3D hydrogel protocols when optimal application conditions have not yet been determined, or when optimization of gel firmness is not needed.

Reagents preparations

Follow instructions indicated in product datasheets for products preparation.

Do not expose CD cell-degradable crosslinker to air and room temperature longer than necessary to avoid oxidation of the thiol-groups and make sure to close caps immediately after each use.

Use culture medium, PBS or physiological solution to prepare your stock cell suspension or other biological sample.

Experimental procedure

IMPORTANT: please read the complete chapter before mixing your reagents.

Hydrogel mix preparation considerations:

Your cell suspension should represent 1/5 of the final gel volume. Accordingly, the final concentration of cells in the complete gel will be 1/5 of your stock cell suspension.

Reagents	Example of reagent volumes in µL		
Thiol reactive RGD degradable polymer	18	36	72
CD cell-degradable crosslinker	2	4	8
Cells suspension	5	10	20
Total in µL	**25**	**50**	**100**

Table 1: Reagents volume to set up a hydrogel using TrueGel3D™ hydrogel CD cell-degradable crosslinker and RGD peptide components

Experimental Procedure:

1. Prepare cell suspension using culture medium, PBS, or any other physiological solution.
2. Add RGD degradable polymer to cell suspension in a reaction tube and mix gently. Add CD cell-degradable crosslinker to the mixture and mix by pipetting gently up and down a few times.
3. Plate the mixture in a culture dish of your choice* before it begins to solidify.

   Note: The mix will remain liquid for 1 to 4 minutes before gel begins to form: make sure to dispense the entire volume of the mixture within this interval.

   * You can use multiwell plates (6-,24- or 96- well) or any kind of sterile culture plate or flask. Non-tissue culture treated polystyrene is recommended. For imaging, we recommend glass slides with cell culture chambers, such as Millicell® EZ SLIDE 8-well glass, sterile (product number PEZGS0816)
4. Incubate the mixture at 370C for 20 minutes. The mixture may also be incubated at room temperature, though gel setup may take longer.
5. Test gel formation by gently touching gel with pipet tip. If no threads of gel pull away from the surface when the tip is withdrawn, the gel is set.
6. Add sufficient culture medium to cover the gel.
7. Place the lid on culture dish or flask and incubate in tissue culture incubator.
8. After one hour of incubation, replace the culture medium.
9. Change the medium as required during cultivation of cells.

Dissolving TrueGel3D™ hydrogel CD cell-degradable crosslinker and RGD peptide with TrueGel3D™ enzymatic cell recovery solution:

You can dissolve TrueGel3D™ Hydrogels containing live or chemically fixed cells by adding TrueGel3D™ enzymatic cell recovery solution to the culture medium. For example, a 30 µL gel can be dissolved with 300 µL of a 1:20 dilution of TrueGel3D™ enzymatic cell recovery solution in medium (30-60 minutes incubation, 37°C). After dissolution of the gel, centrifuge the cell suspension and suspend the pelleted cells in fresh medium or physiological buffer. Repeat this washing procedure once or twice to remove remaining TrueGel3D™ enzymatic cell recovery solution. The removal of enzyme is important if cells are to be embedded again in dextran hydrogels to continue culture. If TrueGel3D™ enzymatic cell recovery solution is not removed completely, it can destabilize the newly set up hydrogel.