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Product Information

TrueGel3D Hydrogel Kits
SLO-PVA, CD cell-degradable crosslinker

Catalog Number **TRUE8**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

TrueGel3D Hydrogel Kits with SLO-PVA polymer are used to set up chemically defined fast gelling hydrogels. Gel stiffness can be customized to match that of the native cell environment. The gel is formed by crosslinking of SLO-PVA polymers with CD cell-degradable crosslinkers. The SLO-PVA polymers contain maleimide groups which decrease the time taken for gel formation. The CD cell-degradable crosslinker is composed of matrix metalloproteinase (MMP)-cleavable peptide (Pro-Leu-Gly-Leu-Trp-Ala), which allows cells to spread and migrate by secreting matrix metalloproteinases (MMP1, MMP3, MMP7, and MMP9). Slow gelling hydrogels can be used in microchannels or syringes.

Extracellular matrix (ECM) proteins (Fibronectin, Laminin) or other bioactive components like growth factors can also be added to the hydrogel mix: please refer to TrueGel3D Slow protocol online for more details.

TrueGel3D Hydrogel with SLO-PVA polymer can be customized by adding TrueGel RGD peptide (Catalog Number TRUERGD) to provide attachment sites for cells. The cells are encapsulated during crosslinking, where they can adhere to the polymer through the RGD peptide and grow within the hydrogel.

Components

- SLO-PVA solution 170 µL
  in phosphate buffer
  Each tube contains 30 mmol/L reactive groups
  Catalog Number TRU-SPVA

- CD cell-degradable crosslinker 200 µL
  lyophilized
  Each tube contains 20 mmol/L reactive groups
  Catalog Number TRU-CD

- TrueGel3D buffer 200 µL
  10× concentrated, pH 7.2
  Catalog Number TRU-B72

- Water 2 × 1,500 µL
  Catalog Number TRUWA

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

CD cell-degradable crosslinker

- Centrifuge the vial to make sure entire material is at the bottom of the tube.
- Add 188 µL of water to make a concentration of 20 mmol/L thiol groups.
- Vortex until all material is dissolved.
- Incubate at room temperature for 5 minutes.
- Vortex and centrifuge the tube.
- CD cell-degradable crosslinker is now ready to use.

Storage/Stability

- The lyophilized powders may be stored unopened in the original bottles at –70 °C for up to one year.
- SLO-PVA may be stored at –70 °C for long term and 4 °C for short term.
- Do not expose the CD cell-degradable crosslinker/RGD peptide to air longer than necessary to avoid oxidation of thiol groups. After reconstitution, it can be stored at –20 °C or –70 °C.
- Buffers are stored at 4 °C for short term (<2 months) and for long term between –20 °C and –70 °C.
- Water can be stored between –70 °C and room temperature.

Procedure

Formation of Hydrogel

All steps are performed in sterile hood and the volume ratio of each component is added as indicated below.

Components	**Without peptide (μL)**	**With Peptide (μL)**
Water	16.6	15.3
TrueGel3D buffer, 10× concentrated, pH 7.2	2.4	2.4
SLO-PVA (30 mmol/L)	2.0	2.5
RGD peptide (20 mmol/L)	–	0.8
Cell suspension	6.0	6.0
CD cell-degradable crosslinker (20 mmol/L)	3.0	3.0
Total	**30.0**	**30.0**

1. Prepare cell suspension using culture medium, PBS, or any other physiological solution.

2. Mix water, 10× TrueGel3D buffer, pH 7.2, and SLO-PVA in a reaction tube and mix well.

3. Add the RGD peptide (if applicable) to the reaction tube containing SLO-PVA and mix immediately to ensure homogenous distribution. Incubate 20 minutes to allow attachment of the RGD peptide to the SLO-PVA polymer.
   Note: If RGD peptide is not used, skip this step.

4. Add the cell suspension to the reaction tube containing the polymer (SLO-PVA) to prepare cell suspension mix.

5. Add the CD cell-degradable crosslinker to the cell suspension mix and pipette for a few times.
   Note: Do not incubate longer than 1 minute because the solution will solidify and cannot be transferred through the pipette.

6. Transfer the solution in a sterile culture dish. Incubate for 25 minutes at room temperature or at 37 °C.

7. Once gel has formed, add the cell culture medium until the gel is covered.

8. Incubate the culture dish in the incubator.

9. Replace the medium after 1 hour.

10. Change the medium as in when required for proper growth of cells.

Reference

1. Knight, C.G. et al., A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metalloproteinases. *FEBS Lett,.* **296**, 263–266 (1992).

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