

Product Information

TrueGel3D Hydrogel Kits

FAST-PVA, CD cell-degradable crosslinker

Catalog Number **TRUE4**

Storage Temperature $-70\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

TrueGel3D Hydrogel Kits with FAST-PVA polymer are used to set up chemically defined fast gelling hydrogels. The gel is formed by crosslinking of FAST-PVA polymers with CD cell-degradable crosslinkers. The FAST-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). Fast gelling hydrogels are used when the application requires fast gelation, as in the case of bioprinting.

TrueGel3D Hydrogel with FAST-PVA polymer can be customized by adding TruGel 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 RGD peptide and grow within the hydrogel.

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

Components

- FAST-PVA solution in phosphate buffer
Each tube contain 30 mmol/L reactive groups
Catalog Number TRU-FPVA 170 μL
- CD cell-degradable crosslinker lyophilized
Each tube contain 20 mmol/L reactive groups
Catalog Number TRU-CD 200 μL

- TrueGel3D buffer, 10 \times concentrated, pH 5.5
Catalog Number TRU-B55 200 μL
- Water
Catalog Number TRUWA 2 \times 1,500 μL

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 non cell-degradable crosslinker is now ready to use.

Storage/Stability

- The lyophilized powders may be stored unopened in the original bottles at $-70\text{ }^{\circ}\text{C}$ for up to one year.
- FAST-PVA should be stored at $-70\text{ }^{\circ}\text{C}$.
Note: Avoid frequent freeze-thaw cycles.
- Do not expose the crosslinker/RGD peptide to air longer than necessary to avoid oxidation of thiol groups. After reconstitution, it can be stored at $-20\text{ }^{\circ}\text{C}$ or $-70\text{ }^{\circ}\text{C}$.
- Buffers are stored at $2\text{--}8\text{ }^{\circ}\text{C}$ for short term (<2 months) and between $-20\text{ }^{\circ}\text{C}$ and $-70\text{ }^{\circ}\text{C}$ for long term.
- Water can be stored between $-70\text{ }^{\circ}\text{C}$ and room temperature.

Procedure

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 5.5 | 2.4 | 2.4 |
| FAST-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 5.5, and FAST-PVA in a reaction tube and mix well.
3. Add the RGD peptide (if applicable) to the reaction tube containing FAST-PVA and mix immediately to ensure homogenous distribution. Incubate 5 minutes to allow attachment of the RGD peptide to the maleimide groups of FAST-PVA.
Note: If RGD peptide is not used, skip this step.
4. Pipette 3.0 μL of CD cell-degradable crosslinker in the sterile culture dish. Do not spread out the crosslinker solution, it needs to be kept as a drop.
5. Add the cell suspension to the reaction tube containing the polymer (FAST-PVA) to prepare cell suspension mix.
6. Transfer 27 μL of cell suspension mix to the culture dish containing 3.0 μL of CD cell-degradable crosslinker and quickly mix by pipetting three times. Incubate for 3 minutes for gel formation.
Note: Gel formation starts after a few seconds of mixing. Test gel formation by gently touching gel with pipette tip, and it should not pull out threads of gel when retracting from the gel surface
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 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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