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Product Information

TrueGel3D Hydrogel Kits
FAST-PVA, PEG non cell-degradable crosslinker

Catalog Number **TRUE5**
Storage Temperature –70 °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 PEG non cell-degradable crosslinkers. The FAST-PVA polymers contain maleimide groups which decrease the time taken for gel formation. 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 to conjugate RGD peptide using TruGel RGD peptide (Catalog Number TRUERGD) prior to the crosslinking step. 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 170 µL
  solution in phosphate buffer
  Each tube contain 30 mmol/L reactive groups
  Catalog Number TRU-FPVA

- PEG non cell-degradable crosslinker 200 µL
  lyophilized
  Each tube contain 20 mmol/L reactive groups
  Catalog Number TRU-PEG

- TrueGel3D buffer, 200 µL
  10× concentrated, pH 5.5
  Catalog Number TRU-B55

- 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

PEG non 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.
- PEG non 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.
- FAST-PVA should be stored at –70 °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 °C or –70 °C.
- Buffers are stored at 2–8 °C for short term (<2 months) and between –20 °C and –70 °C for long term.
- Water can be stored between –70 °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
PEG non 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 PEG non cell-degradable crosslinker into the sterile culture dish. Do no 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 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 as required for proper growth of cells.

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