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Product Information

TrueGel3D Hydrogel Kits
FAST-DEXTRAN, allows cell recovery
CD cell-degradable crosslinker

Catalog Number **TRUE3**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description
The TrueGel3D Hydrogel with FAST-DEXTRAN polymer is used to set up chemically defined fast gelling hydrogel. The gel is formed by crosslinking of FAST-DEXTRAN polymers with CD cell-degradable crosslinkers. The FAST-DEXTRAN polymers contain maleimide groups which decrease the time required for gel formation. The CD cell-degradable crosslinker is composed of a 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-DEXTRAN polymer can be customized by adding 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.

TrueGel3D hydrogel with DEXTRAN polymer can be dissolved by treatment of TrueGel3D enzymatic cell recovery solution (Catalog Number TRUEENZ) to recover cells for post culture analysis.

Components
- FAST-DEXTRAN 170 µL
  lyophilized
  Each tube contain 30 mmol/L reactive groups
  Catalog Number TRU-FDE
- CD cell-degradable crosslinker 200 µL
  lyophilized
  Each tube contain 20 mmol/L reactive groups
  Catalog Number TRU-CD
- TrueGel3D buffer, 200 µL
  10× concentrated, pH 5.5
  Catalog Number TRU-B55
- Water 2 × 1500 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
FAST-DEXTRAN
- Centrifuge the vial to make sure entire material is at the bottom of the tube.
- Add 175 µL of water to make a concentration of 30 mmol/L maleimide groups.
- Vortex until all material is dissolved.
- Incubate the tube on ice for 5 minutes.
- Briefly vortex and centrifuge the tube.
  Note: Keep on ice while in use.

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.
- FAST-DEXTRAN is stored at –70 °C after reconstitution.
- 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.

Procedures

Formation of Hydrogel

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

Table 1

Components of Hydrogel

Components	**Without Peptide** (μL)	**With Peptide** (μL)
Water	16.6	15.3
TrueGel3D buffer, 10× concentrated, pH 5.5	2.4	2.4
FAST-DEXTRAN (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-DEXTRAN in a reaction tube and mix well.

3. Add the RGD peptide (if applicable) to the reaction tube containing FAST-DEXTRAN and mix immediately to ensure homogenous distribution. Incubate 5 minutes to allow attachment of the RGD peptide to the maleimide groups of the polymer.
   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 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-DEXTRAN) 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.

Steps to recover cells

TrueGel3D enzymatic cell recovery solution is used to dissolve the hydrogel matrix.

1. Add 300 μL of 1:20 diluted TrueGel3D enzymatic cell recovery solution to dissolve 25 μL of gel.
   Note: Rate of dissolution is increased if gels are cut into pieces
2. Incubate at 37 °C for 30–60 minutes.
3. Centrifuge the cell suspension and resuspend the pelleted cells in fresh medium or buffer.
4. Repeat step 2 twice to wash the remains of TrueGel3D enzymatic cell recovery solution from the gel components.
5. Cells are now ready to use for post culture analysis or to set up new hydrogel.
   Note: If TrueGel3D enzymatic cell recovery solution is not removed completely, it will destabilize the newly set up hydrogel.

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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