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Product Information

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

Catalog Number **TRUE2**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

TrueGel3D Hydrogel with FAST-DEXTRAN polymer is used to set up a chemically defined fast gelling hydrogel. The gel is formed by crosslinking of FAST-DEXTRAN polymers with PEG non cell-degradable crosslinker. The FAST-DEXTRAN 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-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 the 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, lyophilized 170 µL
Each tube contains 30 mmol/L reactive groups
Catalog Number TRU-FDE

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

TrueGel3D buffer, 10× concentrated, pH 5.5 200 µL
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.

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 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 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.

Procedures

Formation of Hydrogel

All steps are performed in sterile hood andthe volume ratio of each component is added as indicated in Table 1.

Table 1.
Gel Component Volumes

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
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× 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 polymer and mix immediately to ensure homogenous distribution. Incubate 5 minutes to allow attachment of the RGD peptide to the maleimide groups of polymer.
   Note: If RGD peptide is not used, skip step 3

4. Pipette 3.0 μL of PEG non 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 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 gels 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.

Recovery of 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 3 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 destabilizes the newly set up hydrogel.

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