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Product Information

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

Catalog Number **TRUE6**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description
TrueGel3D Hydrogel Kits with SLO-DEXTRAN polymer are used to set up slow gelling hydrogels. It has higher stiffness that can be customized to match that of the native cell environment. The gel is formed by crosslinking of SLO-Dextran polymers with PEG non cell-degradable crosslinker. The SLO-DEXTRAN polymers are used to prepare slow gelling hydrogels, which allow enough time to conveniently manipulate the solution unlike fast gelling hydrogels. Slow gelling hydrogels can be used in microchannels or syringes.

TrueGel3D Hydrogel with SLO-DEXTRAN polymer can be customized by adding TrueGel3D 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 Slow 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
- SLO-DEXTRAN solution 170 µL
  in phosphate buffer
  Each tube contains 30 mmol/L reactive groups
  Catalog Number TRU-SGD

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

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

- 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
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.
- SLO-DEXTRAN may be stored at –70 °C for long term and at 4 °C for short term.
- 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
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-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× TrueGel3D buffer, pH 7.2, and SLO-DEXTRAN in a reaction tube and mix well.

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

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

5. Add the PEG non cell-degradable crosslinker to the cell suspension mix and mix by pipetting a few times.

6. Incubate the solution for 10 minutes at room temperature.
   Note: Do not incubate longer than 10 minutes because the solution will solidify and cannot be transferred through the pipette

7. Resuspend cells by pipetting a few times to ensure uniform distribution in the gel and transfer the solution in a sterile culture dish.

8. Incubate for 50 minutes at room temperature or at 37 °C.

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

10. Incubate the culture dish in the incubator.

11. Replace the medium after 1 hour.

12. Change the medium as in 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 of TrueGel3D Enzymatic Cell Recovery Solution diluted 20-fold with cell culture supernatant 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 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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