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Product Information

TrueGel3D Hydrogel Kits
CD cell-degradable crosslinker and RGD peptide

Catalog Number **TRUE1**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

TrueGel3D hydrogel pre-configured kit is used to prepare a chemically defined hydrogel with stiffness of ~400 Pa (shear modulus) that can be customized to match that of native cell environment.

The TrueGel3D hydrogel is formed by crosslinking of RGD degradable polymer with CD cell-degradable crosslinker. The cells are encapsulated during crosslinking, where they can adhere to the polymer through the RGD peptide and grow within the hydrogel. The CD cell-degradable crosslinker is composed of matrix metalloprotease (MMP)-cleavable peptide (Pro-Leu-Gly-Leu-Trp-Ala), which allows cells to spread and migrate by secreting matrix metalloproteases (MMP1, MMP3, MMP7, and MMP9).

TrueGel3D hydrogel with RGD degradable polymer can be dissolved by treatment of TrueGel3D Enzymatic Cell Recovery Solution (Catalog Number TRUEENZ) to recover cells for post culture analysis.

Components

RGD degradable polymer, lyophilized Catalog Number TRU-RGD	2 × 870 µL
CD cell-degradable crosslinker, lyophilized Catalog Number TRU-CD	200 µL
TrueGel3D reconstitution Buffer Catalog Number TRU-REC	2 × 900 µL
Water Catalog Number TRUWA	600 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

RGD degradable polymer
Note: When stored at –70 °C, warm RGD degradable polymer to room temperature before each use.

- Reconstitute a tube (RGD degradable polymer) with 860 µL of buffer.
- Vortex until all material is dissolved.
- Incubate reconstituted polymer for 1 hour at room temperature.
- Centrifuge briefly; RGD degradable polymer is ready to use.

CD cell-degradable crosslinker

- Reconstitute a tube with 188 µL of water to obtain concentration of 20 mM 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 ready to use.

Storage/Stability

- The lyophilized powders may be stored unopened in the original bottles at –70 °C for up to one year.
- Do not expose the crosslinker to air longer than necessary to avoid oxidation of thiol groups. After reconstitution, it can be stored at –20 °C or –80 °C.
- For short term storage (<1 month) both RGD degradable polymer and reconstitution buffer are stored at 4 °C.
- Water can be stored between –70 °C and room temperature.

Procedures

Formation of Hydrogel

All steps are performed in sterile hood.

1. Prepare cell suspension using culture medium, PBS, or any other physiological solution.
   Note: Cell suspension should be 20% of final volume of the gel.

2. Add RGD degradable polymer to cell suspension in a reaction tube and mix gently. Add CD cell-degradable crosslinker to the mixture and mix by pipetting few times. The volume ratio of each component is added as indicated in Table1.

   Table 1.
   Gel Component Volumes

Components	**Volume parts**
RGD degradable polymer	18
Cell suspension	5
CD cell-degradable crosslinker	2
Total gel volume	25

3. Plate the mixture in a culture dish before it begins to solidify.
   Note: The mix will stay liquid between 1–4 minutes and afterward will begin to form a gel. Make sure to dispense all of the mix during the period after mixing
4. Incubate the mixture at 37 °C for 20 minutes. Alternatively, it can also be incubated at room temperature with a slightly longer incubation period.
5. Test gel formation by gently touching gel with pipette tip. It should not pull out threads of gels when retracting from the gel surface.
6. Add the culture medium to cover the gel.
7. Place the lid on culture dish and incubate for cell cultivation.
8. Replace the medium after 1 hour.
9. Change the medium as needed 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.

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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