TOOLS 3D Culture Plus Kit

For establishing hydrogel 3D cell culture

Contents

Introduction

TOOLS 3D Culture Plus Kit is a comprehensive set of reagents to establish the microenvironments for 3D cell culture and related applications. The polymers included in TOOLS 3D Culture Plus Kit can generate hydrogels at fast gelation rate. The full control of biomolecular modifications and gel stiffness could be achieved easily and efficiently with this ready-to-use kit and allows a great variety of cell culture applications.

Applications

- Spheroid formation assays for tumor cell lines
- Various 3D cell culture-based drug screening platforms.

Materials needed but not supplied with the product

- Cell culture medium suitable for experiment setting
- Culture plates (sterile)
- 1X PBS buffer (sterile)
- Ice box
- (Optional) Thermoconductive tray

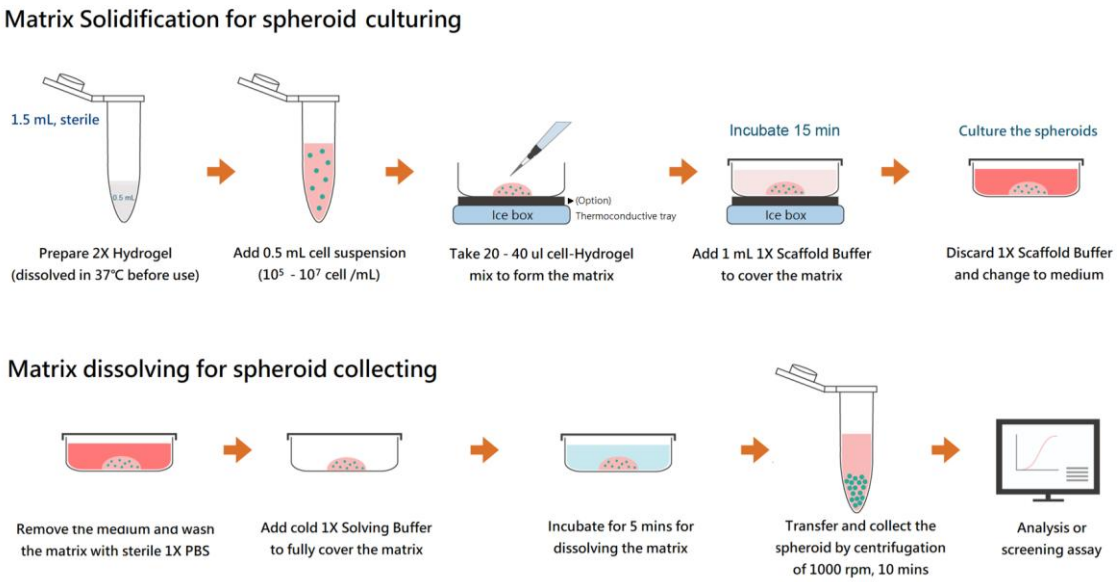
Kit Contents

Components	TTL-3D48 (48 assays)	TTL-3D480 (480 assays)
Hydrogel (2X)	0.5mL/ tube, 2 tubes	0.5mL/ tube, 20 tubes
Scaffold Buffer (10X)	10 mL / BT	50 mL / BT
Solving Buffer (10X)	10 mL / BT	50 mL / BT

Storage

TOOLS 3D Culture Plus Kit should be stored at 4 °C, and aseptic operation practices is essential throughout the preparation and handling process to keep the sterility of the product.

Workflow



Protocol

A. Preparation of the reagents

1. Place Hydrogel in 37°C water bath until it is completely thawed. This process should take at least 10 min.
2. Prepare 1X Scaffold buffer freshly right before use by diluting the 10X Scaffold buffer with cold serum-free culture medium (e.g., serum-free DMEM).

Note: Prepare the 1 X Scaffold buffer with serum-free culture medium only. PBS or any other general salt buffer should not be used to dilute the Scaffold buffer.

3. Prepare 1X Solving buffer freshly by diluting 10X Solving buffer with cold 1X PBS right before use

B. Preparation of 3D cell culture

Note: All the following steps should be performed in laminar flow and the volume ratio of each component is applied as indicated

1. Cool a culture plate/dish. There are two options to proceed:
   a. Directly place the culture plate/dish onto the cracked ice in an ice box at least for 20 mins before use.
   b. Use the thermoconductive tray to cool the culture plate surface rapidly and evenly right before use. The thermoconductive tray should also be pre-cooled beforehand and place onto ice during the process.

Note: Keep the culture plate/dish on the flat surface parallel to the ground.

2. Resuspend 1×10^5~10^7 cells within 1 mL growth medium and then mix with 2X Hydrogel at 1:1 ratio. For example, prepare 1×10^5 cells resuspended in 500 µL growth medium and then mix with 500 µL Hydrogel.

Note: Prepare the cells in appropriate medium and growing conditions. Adherent cells should be about 80% confluency

3. Take the ice-cold culture plate/dish from step 1. Add 20-40 µL cell-Hydrogel suspension mix from step 2 on each well. The mixture should form gel matrix after 5 min.

Note: Check matrix solidification by gently touching gel with pipette tip, and matrix surface should not be pulled out when retracting the tips.

4. Once matrix solidification has formed, add 1 mL of cold 1X Scaffold Buffer to cover the matrix and incubate for 15 min.
5. After incubation, remove the 1X Scaffold Buffer carefully and replace it with culture medium. Change culture medium in the following days.
6. Place the cells at 37°C in CO_2 incubator for 7 to 14 days and observe spheroid formation with microscope. The Culture medium may be changed every other day as required for proper growth of cells.

C. Dissolving 3D Gels for spheroid collection

1. Remove culture medium carefully and wash the matrix with 1X PBS.
2. Remove 1X PBS carefully and apply 1 mL 1X Solving buffer onto matrix for 5 min and incubation at room temperature.
3. Gently mix the solution by pipetting with a 1 mL tip until matrix is completely dissolved.
4. Transfer the spheroid-contained mixture from step 3 into a 1.5 mL tubes. Spin down the spheroids at 1,000 rpm for 10 min. Discard the supernatant and resuspend spheroids in media for assay of interest.

D. Isolation of individual cells from spheroids

Note: To isolate individual cells, spheroid collection described in Procedure B should be performed previously.

1. Add trypsin-EDTA to spheroids and incubate the solution at 37°C. Gently disturb the solution by pipetting until spheroids are dissociated completely.
2. After spheroids are dissociated, add 3 volumes of 1X PBS and spin down cells by centrifugation at 1,000 rpm for 10 min. Discard the supernatant and collect cells for proceeding assays.

Supplementary Information

Spheroid growth of HCT116 cells with TOOLS 3D Culture Plus kit

HCT116 cells were cultured in 3D with TOOLS 3D Culture Plus kit for day 0, day 3 and day 7. The spheroids grew successfully, and the volumes of spheroids increased following days. (Figure 1)

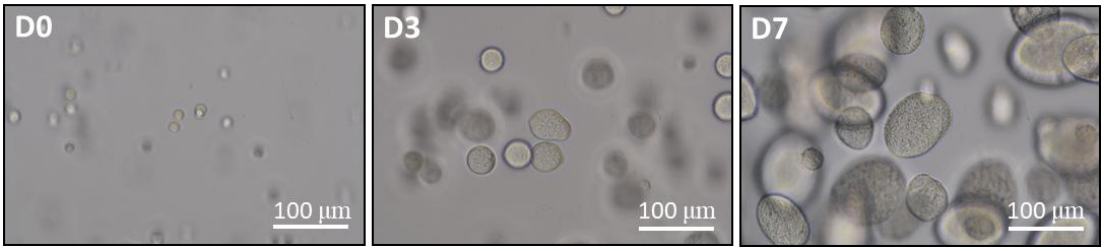


Figure 1. Spheroid growth in TOOLS 3D Culture. (200x magnification)

The product is for research only; it is not for diagnostic or clinical use.