LEADGENE
LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Leadgene® ScaffoldForm 3D Culturing Gel

Cat no. LDG0005RO

Product Overview

Package component

Item	240 assays	120 assays
3D Culturing Gel (2X)	10 vials (0.5 mL)	5 vials (0.5 mL)
Covering Buffer (10X)	2 vials (12.5 mL)	1 bottle (12.5 mL)
Dissociating Buffer (10X)	2 vials (12.5 mL)	1 bottle (12.5 mL)

Product description

The 3D cell culture hydrogel system simulates the microenvironment of human tissue using synthetic biological materials. It is stable, adjustable, easy to operate, and has high biological compatibility, which can be applied in 3D cell culture, tumor formation experiment, tumor angiogenesis and invasion study.

Storage and expiration date of reagents

- Stored at 2-8°C. The diluted buffer solution should be used within one week after opening. It is not recommended to store the 3D Culturing Gel after opening to avoid the effects of repeated freezing and thawing.
- All reagents are stable for two years under proper storage conditions.
- The 3D Culturing gel can maintain its structure in the culture medium for at least 2 weeks.

Procedure

- **Cell seeding and Gelation**

(1) Prepare cells with culture medium at the concentration between 10^5-10^7 cells/mL in 0.5 mL medium.

(2) Place the culture plate on ice for pre-cooling and fully dissolve 0.5 mL Culturing gel in 37°C water bath for 5-10 minutes. After the gel is completely dissolved, mix it with cells and add the mixture into the well.

(3) After 5 minutes, add ice-cold 1X Covering Buffer into the well to submerge the gel dome.

(4) Carefully Replace the 1X Covering Buffer with culture medium after 15 minutes.

(5) Culture cells for 1 to 2 weeks. The Culture medium can be replaced every other day as required for proper growth.

Gel dissolving and Spheroid collection

(1) Remove the culture medium and gently rinse the gel dome with 1X PBS.

(2) Remove 1X PBS and submerge the gel dome with 1X Dissociating Buffer for 5 minutes at room temperature.

(3) Gently pipet the solution until gel dome is dissolved. Add 1X PBS with 3X volume and transfer the solution to 1.5 mL tubes.

(4) Centrifuge at 1000 rpm for 10 minutes. Discard the supernatant and collect the spheroids for experiment or analysis.

(5) To isolate individual cells, add trypsin-EDTA to spheroids and incubate at 37°C. Pipet the solution until spheroids are dissociated and add 1X PBS with 3X volume.

(6) Centrifuge at 1000 rpm for 5 minutes. Discard the supernatant and collect cells for experiment or analysis.

Notes

(1) It is recommended to use 24-well plate for culture, and add 20-40 µL of gel to each well.

(2) To ensure the ease of pipetting, homogeneous gel structure, and prevent the detachment of the gel during culture, it is necessary to fully dissolve the gel in a water

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bath at 37°C before use.

(3) Buffer should be diluted before use. 10X Covering Buffer should be diluted with serum-free DMEM. 10X Dissociating Buffer should be diluted with 1X PBS.

(4) Before adding Covering Buffer into the culture well, user can test gel formation by gently touching gel with pipette tip, and gel thread should not be pulled out when retracting tips from gel surface.

(5) To perform tumor invasion experiment, dilute 1X 3D Culturing Gel with 0.5X Covering Buffer at a ratio of 1:14 (15-fold dilution of 3D Culturing Gel). It is recommended to adjust dilution factor based on the cell type. In addition, to prevent damaging the thin gel layer during Covering Buffer removal, it is recommended to incubate the Buffer covering gel overnight at 4°C and directly add the serum-free cancer cell suspension to the upper chamber of transwell.

(6) The tested 3D Culturing Gel dilution ratios are between 1:4 to 1:14. Please fine-tune the dilution ratio to find the optimal condition for your assay.