

How to Use Mebiol Gel with Multi-well Plate

(continued from
in Instruction manual)

Preparation of Mebiol Gel cell suspension

Cool 10mL of Mebiol Gel solution dissolved in culture medium within 70mL flask and 14mL sterilized centrifuge tube on crashed ice in a beaker (1000mL) .



Transfer required volume (3 ~ 4mL) of Mebiol Gel from the flask to the tube in a clean bench. Remained Mebiol Gel solution can be preserved in a refrigerator or a freezer.

Add 30 ~ 40 μ L of cell suspension (~ 10^5 cell/mL) into Mebiol Gel solution (3 ~ 4 mL) in the centrifuge tube and rotate the tube on ice to mix them.



1

Pour into Multi well Plate

Warm up 24-well plate and overlaying culture medium to 37 beforehand.

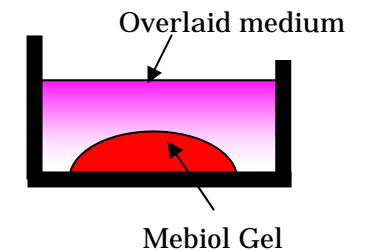
Pour 200 ~ 250 μ L of the cold Mebiol Gel cell suspension (~ 10^3 cell/mL) into center of each well of 24-well plate warmed up to 37 .

For this process, using a large caliber pipette tip such as Rainin Certified™ tips is recommended because Mebiol Gel shows high viscosity.



Mebiol Gel cell suspension in the well gels like island on the plate by warmed up. Not to cover well bottom surface with Mebiol Gel completely is recommended because exposed well surface makes it easier to exchange overlaid medium.

Overlay 400 ~ 500 μ L of culture medium containing phenol red on the island like Mebiol Gel cell suspension at 37 .



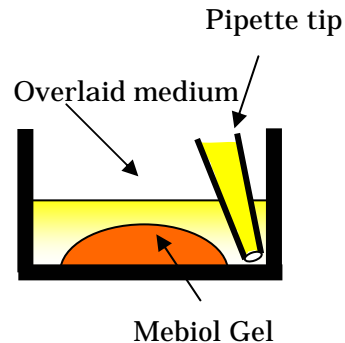
2

Cells can be cultured three-dimensionally in hydrogel state Mebiol Gel at 37 °C in CO₂ incubator.

Culture Observation and Medium Exchange

During cultivation, cells can be observed by optical microscope, however, quick observation and keeping warm the plate are required to prevent Mebiol Gel from dissolving in culture medium by lowering temperature.

Exchange overlaid medium when the medium color turned to yellow (low pH). Suck up the yellow medium by pipette contacting the tip end onto the exposed well surface. Overlay 400 ~ 500 μL of culture medium containing phenol red on the island like Mebiol Gel cell suspension at 37 °C. This medium exchange procedure should be performed quickly and temperature should be kept at 37 °C as much as possible.



Cell Recovery and Passage

To recover cells after cultivation, cool the multi well plate in a refrigerator or on ice and shake gently. By cooled down, the Mebiol Gel is dissolved and diluted in the overlaid culture medium. At this diluted concentration, Mebiol Gel does not gel even above the sol-gel transition temperature. (Adding ca.400 μL of saline to each well reduces viscosity more and makes cell recovery easier.) Transfer the cell suspension in the well to a centrifuge tube and precipitate cells by centrifugation (500 ~ 1,000rpm, 2 ~ 3min) at room temperature.

Passage can be performed by repeating the procedure from .

Manufacturer

Mebiol Inc. R&D Center

Advanced Research Center for Science and Engineering,
Waseda University

3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan

Phone :+81-3-5286-3121, Fax No :+81-3-3209-0336

e-mail :contact@mebiol.co.jp