2003.4.

Instruction for Mebiol Gel®

Features of Mebiol Gel®

An aqueous solution of Mebiol Gel is fluid liquid (sol state) at low temperatures (0˚C～15˚C), however, it turns into an elastic hydrogel (gel state) at temperatures higher than room temperature (25˚C). It is possible to mix it with various drugs or culture medium at the sol state.

The sol-gel transformation of Mebiol Gel occurs fully thermoreversible. Elasticity of the hydrogel increases with temperature increase and is appropriate for three-dimensional culture of cells/tissues at around 37˚C.

Cells/tissues in the gel is clearly observed through optical microscope during cultivation at 37˚C owing to great transparency of Mebiol Gel.

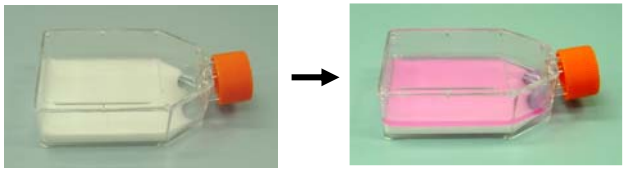
Fibroblast is alive but does not grow in Mebiol Gel therefore other aiming cells can be grown selectively.

Cultured cells/tissues can be recovered easily from Mebiol Gel by lowering temperature without any damage on cells/tissues.

Specifications : MB-10 Mebiol Gel®
Lyophilized (EO sterilized) 10 ml
Sol-gel transition temperature: ca.20˚C

How To Use Mebiol Gel®

1.) Open package in a clean bench and add 10mL culture medium to lyophilized Mebiol Gel in a flask.



2.) Close the flask cap tightly and lay it in a refrigerator (2～10˚C) for about three hours. Lyophilized Mebiol Gel absorbs culture medium slowly.

3.) Dissolve Mebiol Gel in culture medium by shaking occasionally the flask gently with keeping it at low temperature. Usually it takes for one day to dissolve completely. Warming to 37˚C on and off for short period (ca. 1 min) can accelerate dissolution.

After dissolving, settle the solution in a refrigerator (2～10˚C) to eliminate bubbles. Complete elimination of bubbles may take a couple of days.

4.) Add cells/tissues into sol state Mebiol Gel at low temperature (2～10˚C) and then warm it up to 37˚C in CO_2 incubator so that cells/tissues can be cultured three-dimensionally in Mebiol Gel at hydrogel state.

5.) To recover cells/tissues after cultivation, cool Mebiol Gel containing cultured cells/tissues to liquefy it and dilute it with 30～40mL of cold saline or medium. This dilution lowers viscosity of Mebiol Gel and prevents gelation even above the sol-gel transition temperature. Suspended cells/tissues can be easily recovered by centrifugation.

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Information

- Do not use Mebiol Gel® for patients or medical diagnosis. Mebiol Gel® is distributed only for research on *in vitro* cell/tissue culture.

- Do not resterilize Mebiol Gel® to avoid deterioration.

- Mebiol Gel® is packaged with oxygen scavenger in a gas barrier film. After open the package, dissolve Mebiol Gel® in culture medium promptly and keep a solution in a refrigerator. To use the solution within one month is strongly recommended.

For research use only; not for use as a diagnostic

Manufactured by R&D Center Mebiol Inc.

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618

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How to Use Mebiol Gel® with Multi-well Plate

(continued from 4.) in Instruction manual)

Preparation of Mebiol Gel cell suspension

1.) 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).

2.) 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.

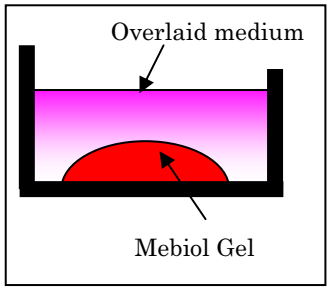
3.) Add 30～40μL of cell suspension (～10^5 cell/mL) into Mebiol Gel solution (3～4mL) in the centrifuge tube and rotate the tube on ice to mix them.

Pour into Multi well Plate

4.) Warm up 24-well plate and overlaying culture medium to 37˚C beforehand.

5.) Pour 200～250μL of the cold Mebiol Gel cell suspension (～10^3 cell/mL) into center of each well of 24-well plate warmed up to 37˚C. For this process, using a large caliber pipette tip such as Rainin Certified™ tips is recommended because Mebiol Gel shows high viscosity.

6.) 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.

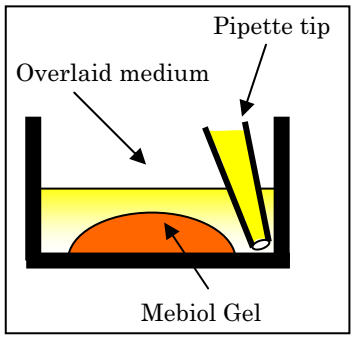


7.) Overlay 400～500μL of culture medium containing phenol red on the island like Mebiol Gel cell suspension at 37˚C.

8.) Cells can be cultured three-dimensionally in hydrogel state Mebiol Gel at 37˚C in CO_2 incubator.

Culture Observation and Medium Exchange

9.) 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.



10.) 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 400～500μL 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

11.) 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.400μL 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.

12.) Passage can be performed by repeating the procedure from 3.).

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http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618