COSMO BIO CO., LTD.

Mebiol Gel®
PNIPAAm-PEG 3D Thermoreversible Hydrogel

Cat. No. MBG-PMW20-1001/ MBG-PMW20-1005/MBG-PMW20-1020/ MBG-PMW20-5001/MBG-PMW20-5005/MBG-PMW20-5020

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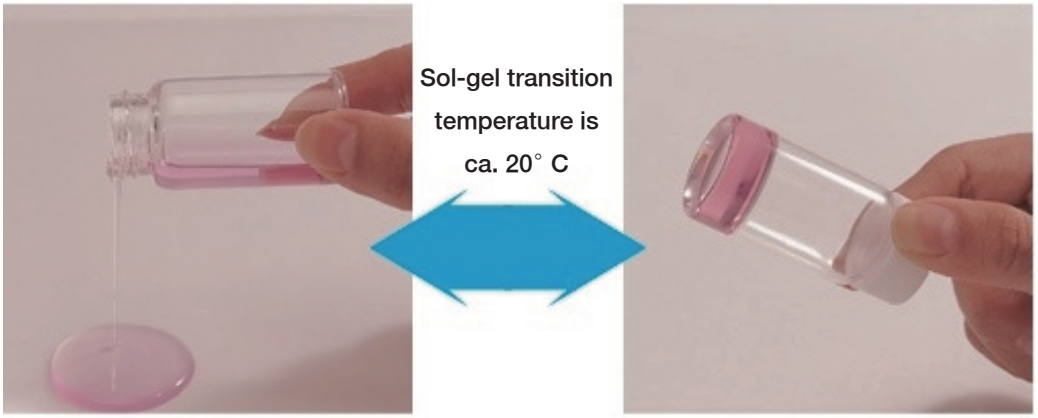
【 I 】 Background

Hydrogels are a diverse class of polymeric materials characterized by their network-like structure and high water content. Hydrogels of many kinds have found a wide variety of applications in medicine and life science research weighted towards, but not at all limited to three-dimensional cell culture, tissue engineering, and drug delivery. Properties highly favorable to cell culture and tissue engineering applications prompted the commercialization of Mebiol Gel®, a copolymer of poly(N-isopropylacrylamide) and poly(ethylene glycol) (PNIPAAm-PEG) for research purposes in the early 2000's.

Mebiol Gel®'s defining feature, in contrast to other commercially available hydrogels, is its temperature reversible sol-gel transition. When cooled, Mebiol Gel® is a sol (handles like a liquid) but becomes a rigid hydrogel at higher temperatures. In practice, this means extremely easy cell handling. Cultures are seeded into cooled Mebiol Gel® and recovered conveniently by cooling the culture vessel and centrifugation. In the gel state, the highly lipophylic environment of the Mebiol Gel® presents an efficient niche for cell proliferation, cell communication, gas and mass exchange, and protection of cells and tissue from shear forces.



Low Temperature (sol) High Temperature (gel)

For research use only, Not for diagnostic use.
Please read this manual thoroughly before use.

【 II 】 Features

- Easy handling
- Non-toxic, biocompatible
- 100% synthetic, pathogen free
- High transparency for cell observation
- Proven performance.

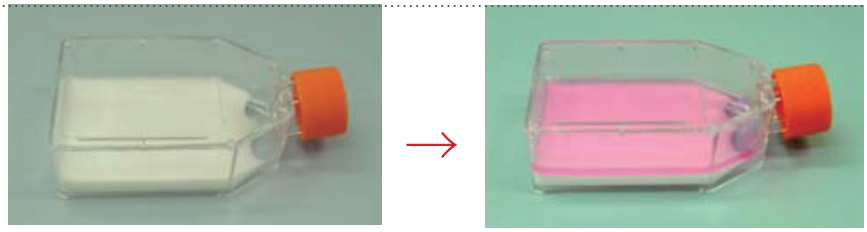
【 III 】 Applications

- Stem cell and pluripotent stem cell culture, expansion, and differentiation
- Spheroid culture
- Cell implantation
- Organ and Tissue Regeneration
- Drug Delivery
- Non-cell culture application

【 III 】 Experimental Procedures

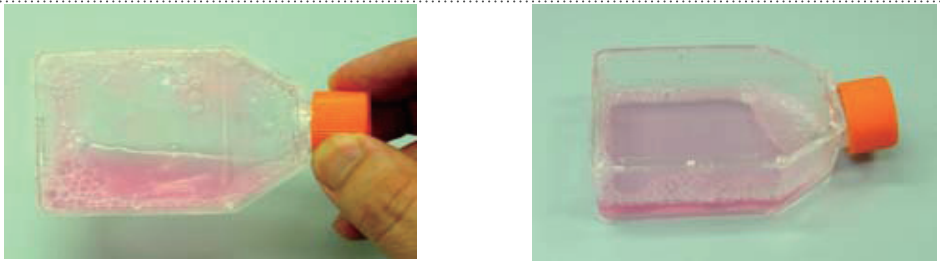
- How to use Mebiol Gel® -

1 Open package on a clean bench and add 10mL of culture medium to lyophilized Mebiol Gel® in the flask.



2 Close the flask cap tightly and place it in a refrigerator (2-10˚C) for approximately 3 hours. Lyophilized Mebiol Gel will absorb the culture medium slowly.

3 Dissolve Mebiol Gel® in culture medium by occasionally shaking the flask gently while keeping it at low temperature. Usually it takes about 1 day for the gel to dissolve completely. After the gel has dissolved, settle the solution in a refrigerator (2-10˚C) to eliminate bubbles. Complete elimination of bubbles may take a couple of days. Warming to 37˚C on and off for short period (ca. 1 min) can accelerate dissolution.



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

To recover/collect cells/tissues

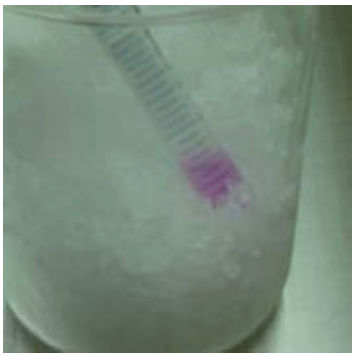
5 To recover cells/tissues after cultivation, cool Mebiol Gel® containing cultured cells/tissues to liquefy it. Dilute it with 30-40ml of cold saline or medium. This dilution lowers the viscosity of Mebiol Gel® and prevents gelation even above the sol-gel transition temperature. Suspended cells/tissues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

Use Mebiol Gel® with a Multi-well Plate

1 Cool 10ml of Mebiol Gel® solution dissolved in culture medium in a 70ml flask and 14ml sterilized centrifuge tube on ice in a beaker (1L)

2 Transfer required volume (3-4ml) of Mebiol Gel from the flask into the tube on a clean bench. The remaining Mebiol Gel® solution can be stored in the fridge or freezer.

3 Add 30-40 ul of cell suspension (～ 10^5 cells/ml) into Mebiol Gel® solution (3-4ml) in the centrifuge tube and mix by rotating the tube on ice.



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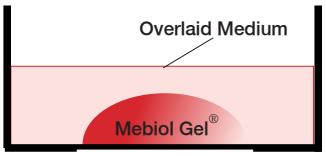
Pour into Multi-well Plate

4 Warm up the 24-well plate and overlaying culture medium to 37°C in advanced.

5 Pour 200-250ul of the cold Mebiol Gel® cell suspension (～ 10^3cell/ml) into the center of each well of a 24-well plate warmed up to 37°C. For this process, usage of a large caliber pipette tip such as Rainin Certified™ tips are recommended as Mebiol Gel® has high viscosity.

6 Mebiol Gel® cell suspension in the well gels like islands on the plate when warmed up. Complete coverage of well bottom surface with Mebiol Gel® is not recommended as exposed well surfaces enable easier overlaid medium exchange.

7 Overlay 400-500ul of culture medium containing phenol red on the island as in Mebiol Gel® cell suspension at 37°C

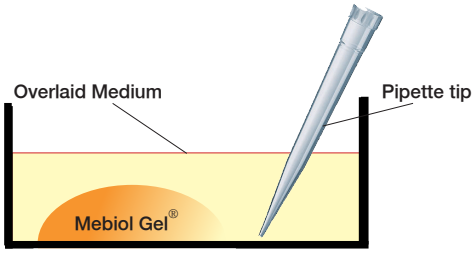


8 Cells can be cultured three-dimensionally in Mebiol Gel® in its hydrogel state at 37° C in an CO_2 incubator.

Culture Observation and Medium Exchange

9 During cultivation, cells can be observed by an optical microscope, however, to prevent Mebiol Gel® from dissolving in culture medium when lowering the temperature, observation must be carried out quickly and the plates must be kept warm.

10 Exchange overlaid medium when the medium color has turned yellow (low pH). Pipette out the yellow medium by 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 close as possible.



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Culture Recovery and Passage

11 To recover cells after cultivation, cool the multi-well plate in a refrigerator or on ice and shake gently. By cooling down, Mebiol Gel® is dissolved and diluted in the overlaid culture medium. At this diluted concentration, Mebiol Gel® does not become gel even above the sol-gel transition temperature. (Adding ca.400µL of saline to each well further reduces viscosity 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 Cell passage can be performed by repeating the procedure from 3.).

【NOTE】

- Expiry date is 1 year after date shipped.
- 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 an oxygen scavenger in a gas barrier film. After opening the package, dissolve Mebiol Gel® in culture medium promptly and keep the solution in a refrigerator.
- Usage of solution within one month is strongly recommended.

Manufactured by R&D Center Mebiol Inc.

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