

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

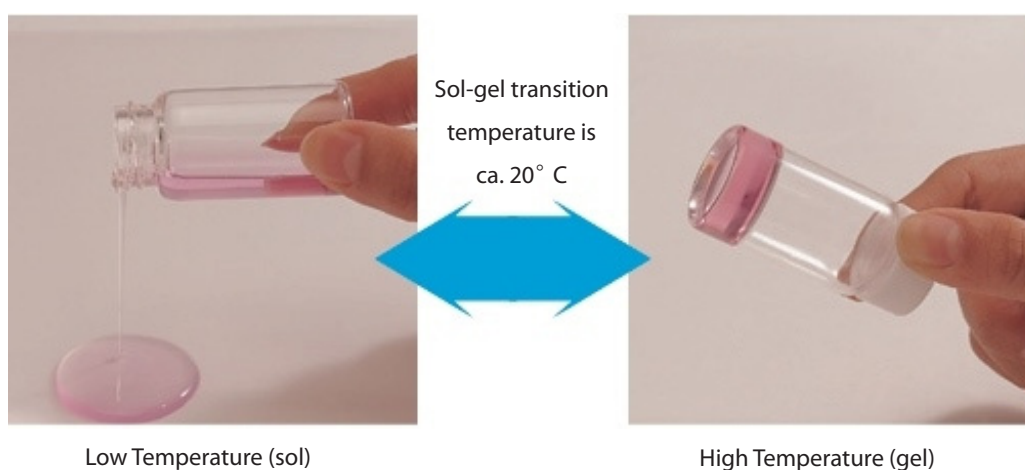
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【1】 Background

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.





【 II 】 Features

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

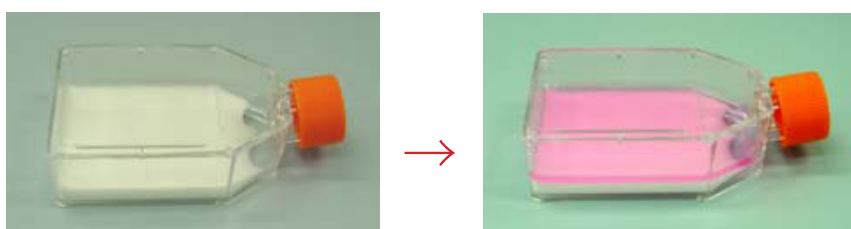
【 III 】 Applications

- ◆ 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® -

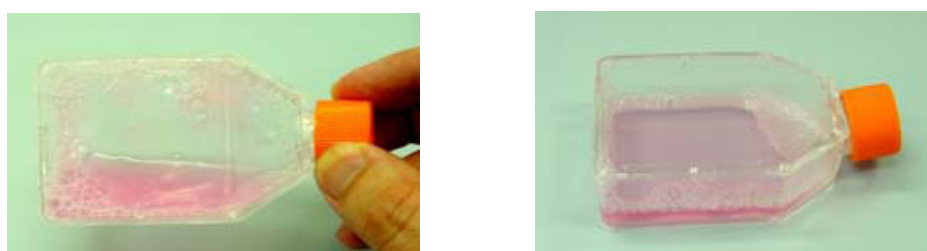
- 1 Open package on a clean bench and add 10mL of culture medium to lyophilized Mebiol Gel® in the flask.



- 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 very gently (do not use a shaker) 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.





- 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 CO₂ 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 (~ 10⁵ cells/ml) into Mebiol Gel[®] solution (3-4ml) in the centrifuge tube and mix by rotating the tube on ice.

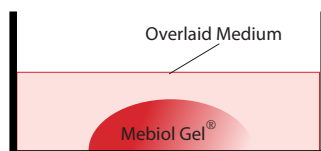


Pour into Multi-well Plate

- 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 ($\sim 10^3$ cell/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.



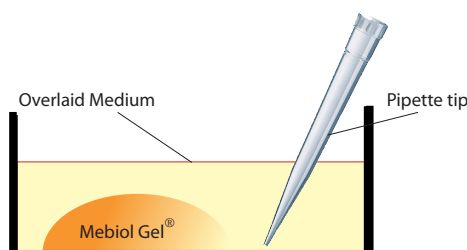
- 6 Mebiol Gel[®] cell suspension in the well gels like islands on the plate when warmed up. Complete coverage of well bottom surface with Mebiol Gel[®] is not recommended as exposed well surfaces enable easier overlaid medium exchange.
- 7 Overlay 400-500ul of culture medium containing phenol red on 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 CO₂ 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.



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.
- ◆ The gel color in this manual is pink to make it easy to look, but the actual product is clear and colorless.
- ◆ Do not use "pink tablet (oxygen detection agent)" and "Oxygen and Moisture Absorbent" that are supplied with the product. They are not parts of the product (see Fig. below).



Manufactured by R&D Center Mebiol Inc.