

Mebiol Gel[®]

PNIPAAm-PEG 3D Thermoreversible Hydrogel

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

Last Updated: 2016/03/29

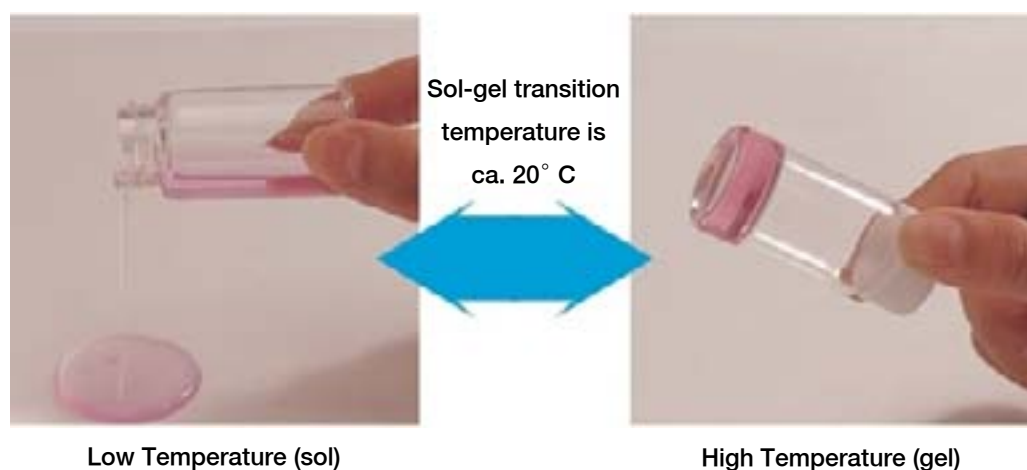
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	Cat. No.	Content quantity	Amount of medium to be added
10 mL type	MBG-PMW20-1001	1 g	10 mL
	MBG-PMW20-1005		
50 mL type	MBG-PMW20-5001	5 g	50 mL
	MBG-PMW20-5005		

【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.

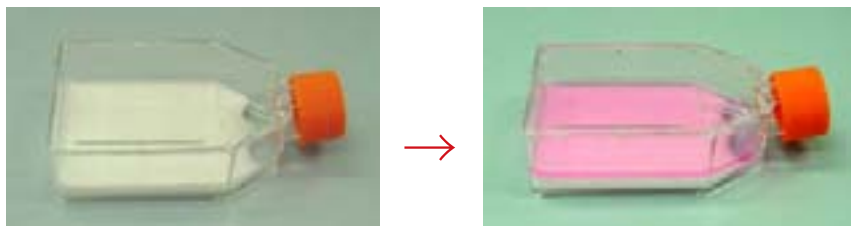
【 III 】 Applications

- ◆ 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 10 mL for 10 mL type and 50 mL for 50 mL type of culture medium respectively to lyophilized Mebiol Gel® in the flask. The final concentration of Mebiol Gel is 10% (w/v).



- 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 30-40 mL (150-200 mL) 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 10 mL(50 mL) of Mebiol Gel® solution dissolved in culture medium in a flask and sterilized centrifuge tube on ice in a beaker (1L)



- 2** Transfer required volume (3-4 mL) 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 ($\sim 10^5$ cells/mL) into Mebiol Gel® solution (3-4 mL) in the centrifuge tube and mix by rotating the tube on ice.

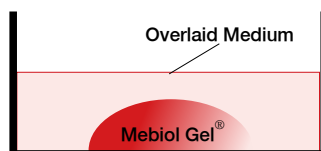


Pour into Multi-well Plate

- 4 Warm up the 24-well plate and overlaying culture medium to 37°C beforehand.
- 5 Pour 200-250 uL 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.



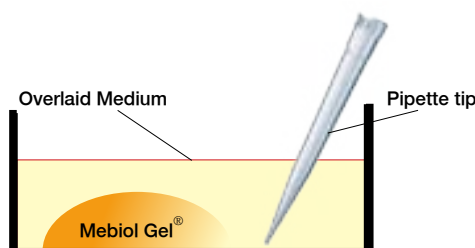
- 6 Mebiol Gel® cell suspension in the well gels like island on the plate when warmed up. Not to cover well bottom surface with Mebiol Gel® completely is recommended as exposed well surface makes it easier to exchange overlaid medium.
- 7 Overlay 400-500 uL of culture medium containing phenol red on 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 CO₂ incubator.

Culture Observation and Medium Exchange

- 9 During cultivation, cells can be observed by an 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 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

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.400 μ L 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 (500-1,000 rpm, 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.