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3D Cell Culture Substrate
MatriMix (511)
Product-Instruction Manual

MatriMix (511) is comprised of three components: Solution A (DMEM, Laminin 511E8 fragment/hyaluronan cross-linked product), Solution B (sodium bicarbonate), and Solution C (mixture of pepsin-solubilized type I/III collagen and acid-extracted type I collagen). Before culturing, the three solutions are mixed and formed a gel at 37°C. Cells can be cultured in or on gel.

Contents

1. 3.6 mL of solution A (1.85x DMEM[*1], laminin 511 E8 fragment/hyaluronan cross-linked)
2. 1.0 mL of solution B (2.5% sodium bicarbonate)
3. 3.0 mL of solution C (5.0 mg/mL collagen[*2])

*1 The DMEM concentration becomes 1x after mixing the three solutions

*2 Mixture of pepsin extracted type I/III collagen and acid-extracted type I collagen

Storage and expiration date

Store at 2-8°C, avoid freezing. Use immediately after mixing solutions[*3].
The expiration date is 6 months after manufacturing without opening the tubes.

*3 After mixing each solution, a gradual increase in pH may affect the gelation rate and fiber formation.

[Notes]
This product is for research use only. It should not be used for humans. Avoid accidental ingestion or contact with skin.
If irritation occurs, wash immediately with large amounts of water and seek medical attention.

Instructions for use

I. Preparation of MatriMix solution

The mixed solution cannot be stored long, so it should be prepared before each use.
To change the collagen concentration of Solution C, please read “III. How to dilute Solution C, collagen solution”.

(1) Calculate the volume ratio to be 5.4:0.6:4.0 (Solution A: Solution B: Solution C = 5.4:0.6:4.0) according to the amount used.

(2) Add Solution B to the aliquoted Solution A[*4]. Then add solution C[*5]. The operation should be performed at low temperature.

*4 When Solution B is added to Solution A, the color changes from grayish blue-green to pink.

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*5 Since the collagen solution in liquid C is highly viscous, do not remove the pipette tip from the liquid surface too early when pipetting. If the tip is pulled up too quickly, air will enter the tip end. After adding collagen solution, mix slowly and thoroughly.

(3) Store at low temperature until ready for use.

II. "In Gel" Embedded culture

The following is an example of culturing cells in a 12-well plate or a multi-well plate or dish with a larger bottom area.

(1) Preheat the culture vessel in a CO_2 incubator set at 37°C.
(2) Prepare the required number of cells.
(3) Centrifuge the cell suspension and keep the cell pellet on ice. To avoid dilution of the MatriMix solution, remove as much of the medium as possible after the centrifugation.
(4) Dispense 150-250 µL of the MatriMix solution prepared in **I. Preparation of MatriMix solution** above and gently suspend the cells to avoid the formation of air bubbles.
(5) Place a dome-shaped () drop of MatriMix solution near the center of the culture surface.
(6) Allow to gel in a CO_2 incubator set at 37°C for at least 30 minutes.
*Before gelation, move the culture plate gently and carefully.
*When gelation occurs, the color of the gel becomes slightly cloudy.
If gelation does not occur, extend the incubation time.
(7) Gently add medium along the well wall to prevent the gel detachment. Move the plate slowly into the incubator.

Adjust the protocol according to the culture vessel used and the application. Please note that if the volume of MatriMix solution is too small or the height of the gel is too low, cells may fall to the dish surface and cannot be cultured three-dimensionally. If the cells easily fall to the dish surface, try a sandwich culture method in which cells are seeded on a pre-made gel.

III. How to dilute solution C, collagen solution

To adjust gel stiffness, Solution C, collagen solution, can be diluted up to x1/2.

(1) Prepare cold sterile water.
(2) Dispense sterile water into a new tube to achieve the volume ratio set in the maximum 1/2-fold dilution range.
(3) Dispense the required volume of Solution C and mix thoroughly with cold sterile water on ice.
(4) Prepare MatriMix solution according to **I. Preparation of MatriMix solution** above.

If you need a higher concentration of collagen, or want to mix other molecules (such as growth factors or other extracellular matrix components), please contact us at MatriMix@nippi-inc.co.jp.

IV. Other Information

In addition to the culture method described in **II. "In Gel" Embedded culture**, sandwich culture and culture on gel are also available. If you have any questions, please refer to the MatriMix website (https://matrimix.nippi.bio/) for FAQs or contact us at MatriMix@nippi-inc.co.jp.