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Product Information

HydroMatrix™ Peptide Cell Culture Scaffold

Catalog Number **A6982**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

HydroMatrix™ is a peptide nanofiber 3-dimensional scaffold that promotes cell growth and migration. HydroMatrix utilizes specific peptides that self-assemble from fluid precursors into highly crosslinked peptide hydrogels in response to increases in temperature or ionic strength. Induction of the rapid solution-gel transformation of this peptide solution generates a peptide nanofiber scaffold that has cell culture and tissue engineering applications.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The product is supplied as a lyophilized powder. A 1% (w/v) Stock Solution (10 mg/ml) may be prepared by addition of sterile water. The 1% (w/v) Stock Solution will result in a rigid gel. A 0.5% (w/v) Working Solution results in a softer gel that is suitable for cell migration and angiogenesis. Working Solution concentrations as low as 0.15–0.25% are suitable for many cell types, including neurons. It is recommended for most applications to start with a 0.25% (w/v) Working Solution (2.5 mg/ml). Solutions must be kept on ice to prevent premature formation of the hydrogel.

In aqueous solution, HydroMatrix has a pH of ~2.5; therefore, it must not be added directly to cells. The addition of buffer or medium is required prior to the addition of cells. For cell encapsulation studies, addition of iso-osmotic sucrose will help protect the cells until a physiological pH is obtained.

Formation of the peptide hydrogel occurs with increases in temperature or ionic strength. Therefore, stock and working solutions must be kept on ice and do not add buffer or medium until ready to form the hydrogel.

Decrease the viscosity of the 1% (w/v) Stock Solution by vortexing or sonication for 10 minutes each time the Stock Solution is used. Remove any air bubbles by brief centrifugation.

Once buffer or medium is added and the hydrogel forms, do not attempt to mix the gel any further as this will destroy the hydrogel integrity. Manipulation of the cells and addition of medium should be carefully performed to avoid disruption of the scaffold. Add medium, growth factors, etc. to the side of the well and let them enter the well via gravity followed by very gentle movement of the plate to facilitate mixing.

Storage/Stability

Store the lyophilized product at –20 °C and it is stable for many years at that temperature. Storage at –70 °C extends the life of the hydrogel considerably.

The 1% (w/v) Stock Solution (10 mg/ml) should be stored at –20 °C and is stable for 3–6 months.

Procedures

<u>Plating of Cells on HydroMatrix-Coated Plates</u>

1. Determine the optimal concentration for the particular application and dilute the 1% (w/v) Stock Solution with distilled water. Keep the Working Solution on ice until ready to use.

2. Add the Working Solution to the cell culture well. See Table 1 for recommended volumes. Initiate gel formation by adding 1–2 volumes of medium to the side of each well.

Table 1.
Recommended Volumes of the Working Solution

Plate Size	Volume of Working Solution
96-well plate	75 µl per well
24-well plate	500 µl per well
6-well plate	2.4 ml per well

3. Incubate the plate at 37 °C for 1 hour to allow the gel to form. Then carefully change the medium twice over 1–2 hours and allow the gel to incubate at 37 °C during this time. Keep the gelled plate at 37 °C for no more than 8 hours before use.

4. Add desired number of cells in medium to the top of the hydrogel.

Plating of Cells on HydroMatrix-Coated Inserts

1. Determine the optimal concentration for the particular application and dilute the 1% (w/v) Stock Solution with distilled water. Keep the Working Solution on ice until ready to use.

2. Add enough medium to the lower chamber of each insert so the medium level is just touching the bottom of the insert.

3. Add the Working Solution to the cell culture insert. See Table 2 for recommended volumes. Initiate gel formation by adding 1–2 volumes of medium to the side of each well.

Table 2.
Recommended Volumes of of the Working Solution

Insert Size	Volume of Working Solution
96-well plate	35 µl per well
24-well plate	250 µl per well
6-well plate	1.0 ml per well

4. Incubate the insert at 37 °C for 1 hour to allow the gel to form. Then carefully change the medium twice over 1–2 hours and allow the gel to incubate at 37 °C during this time. Keep the gelled insert at 37 °C for no more than 8 hours before use.

5. Add desired number of cells in medium to the top of the hydrogel. Add additional medium below the insert.

Cell Encapsulation in Cell Culture Plates

1. Prepare a 2× Working Solution by diluting the 1% (w/v) Stock Solution with a sterile 20% sucrose solution and water to a 2× working concentration of HydroMatrix in 10% sucrose. For example, to encapsulate cells in a final HydroMatrix concentration of 0.25% (w/v), generate 1 ml of a 2× Working Solution by adding 500 µl of sterile 20% sucrose solution, 250 µl of 1% (w/v) Stock Solution, and 250 µl of sterile water. Keep the 2× Working Solution on ice until ready to use.

2. Centrifuge cells and resuspend the cells in 10% sterile sucrose at twice the final desired cell concentration. There should be as little medium left as possible in order to avoid premature gel formation.

3. Laminin or collagen can be added to the 2× Working Solution prior to or after adding the cell suspension. Add laminin or collagen to a final concentration of 1–5 µg/ml. Fibronectin cannot be added to the 2× Working Solution, but can be added to the medium after encapsulation.

4. Add the 2× Working Solution to the 2× cell/sucrose mixture. For example, to make 500 µl of plating solution, add 250 µl of 2× Working Solution and 250 µl of 2× cell/sucrose mixture.
Note: It is recommended to mix only enough for a one plate at a time.

5. Plate the mixture immediately to the middle of the well.

6. Repeat until cells have been plated in all wells.

7. Initiate gel formation by adding 1–2 volumes of medium to the side of each well.

8. Incubate the plate at 37 °C for 1 hour to allow the gel to form. Then carefully change the medium twice over 1–2 hours and allow the gel to incubate at 37 °C during this time. Keep the gelled plate at 37 °C for no more than 8 hours before use.

Cell Encapsulation in Cell Culture Inserts

1. Determine the optimal concentration for the particular application and dilute the stock solution with distilled water. Keep the diluted Hydrogel solution on ice until ready to use.

2. Add enough medium to the lower chamber of each insert so the medium level is just touching the bottom of the insert.

3. Prepare a 2× Working Solution by diluting the 1% (w/v) Stock Solution with a sterile 20% sucrose solution and water to a 2× working concentration of HydroMatrix in 10% sucrose. For example, to encapsulate cells in a final HydroMatrix concentration of 0.25% (w/v), generate 1 ml of a 2× Working Solution by adding 500 µl of sterile 20% sucrose solution, 250 µl of 1% (w/v) Stock Solution, and 250 µl of sterile water. Keep the 2× Working Solution on ice until ready to use.

4. Centrifuge cells and resuspend the cells in 10% sterile sucrose at twice the final desired cell concentration. There should be as little medium left as possible in order to avoid premature gel formation.

5. Add the 2× Working Solution to the 2× cell/sucrose mixture. For example, to make 500 µl of plating solution, add 250 µl of 2× Working Solution and 250 µl of 2× cell/sucrose mixture.
   Note: It is recommended to mix only enough for a one plate at a time.

6. Plate the mixture immediately to the middle of the well.

7. Repeat until cells have been plated in all wells.

8. Initiate gel formation by adding 1–2 volumes of medium to the side of each well.

9. Incubate the plate at 37 °C for 1 hour to allow the gel to form. Then carefully change the medium twice over 1–2 hours and allow the gel to incubate at 37 °C during this time. Keep the gelled insert at 37 °C for no more than 8 hours before use.

Cell Recovery and Passaging

1. Pipette the medium up and down to disrupt the HydroMatrix hydrogel. Transfer material to a centrifuge tube. Rinse the well with PBS to remove remaining cells and gel, and add to the centrifuge tube.

2. Centrifuge at 200–500 × *g* for 5 minutes. Discard the supernatant. Resuspend the pellet containing cells and gel fragments in 5 ml of PBS.

3. Centrifuge at 200–500 × *g* for 5 minutes. Resuspend the pellet with dissociation solution (trypsin-EDTA or non-enzymatic) for 1–5 minutes.

4. Add an equal volume of medium and centrifuge at 200–500 × *g* for 5 minutes. There may be residual gel attached to some cells, which cannot be removed. Replate or analyze cells as desired.

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