GIBCO
invitrogen cell culture

AlgiMatrix™ 3D Culture System

Store at 15°C to 30°C

Product Name	Catalog no.	Components
AlgiMatrix™ 3D Culture System 6-well plates	A10982-01 A10982-02	1 plate + 50 mL Firming Buffer 4 plates + 50 mL Firming Buffer
AlgiMatrix™ 3D Culture System 24-well plates	12684-023 12684-049	1 plate 4 plates
AlgiMatrix™ 3D Culture System 96-well plates	12684-015 12684-031	1 plate 5 plates
AlgiMatrix™ Firming Buffer	A10915-01	50 mL
AlgiMatrix™ Dissolving Buffer	A11340-01	50 mL

Description

The AlgiMatrix™ 3D Culture System is an animal origin-free bioscaffold that facilitates three-dimensional (3D) cell culture. Each bioscaffold is an alginate sponge with a pore size of ~50–200 µm. Cells grown in this sponge more closely match normal cell morphology and behavior, providing an excellent solution for 3D cell-culture models in many research fields, such as toxicology, drug development, cancer research, and tissue engineering. AlgiMatrix™ bioscaffolds are supplied lyophilized in sterile plate wells and are stable at room temperature.

The AlgiMatrix™ 3D Culture System is suitable for many cell-based screening and drug discovery procedures, including Multicellular Tumor Spheroid Assays (MCTS), hepatocyte and cardiomyocyte organogenesis studies, co-culture studies, high-throughput drug screening, and embryonic stem-cell 3D differentiation studies.

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Setting Up AlgiMatrix™ 3D Cell-Culture Experiments

- Plan to use all the wells of each plate at the same time. If empty wells are left un-inoculated in a humidified incubator, they will absorb moisture and lose shape, which limits absorption of cells.
- Select either an upright or inverted lab microscope to view spheroids. Upright microscopes may produce better visualization, but, depending upon the focal length of the lens, may require plate lid removal, limiting this to post-culture observation.
- Perform all procedures aseptically, in a laminar flow hood.
- Optimizing culture inoculation and incubation conditions may improve performance.

Tips for Using AlgiMatrix™

- In general, cell medium should be exchanged at least as frequently as with 2D culture. Lower cell densities may enable less frequent medium exchange. Do not exceed 5 days without medium exchange. Requirements vary by cell type and application.
- To observe the cells inside or on top of the AlgiMatrix™ sponge, decrease the medium volume or relocate the sponge to a dry well. Sponges can be inverted for better observation of cells at the top of the sponges.
- Avoid using phosphate-buffered saline solutions with AlgiMatrix™. PBS softens the sponge and leads to a cloudy culture medium.

AlgiMatrix™ Firming Buffer

AlgiMatrix™ Firming Buffer is included with 6-well plates and can be purchased separately for 24-well and 96-well plates. It is an isotonic, neutral-pH buffered solution designed to increase the firmness of the bioscaffold. Firmer sponges are less susceptible to pore expansion and contraction by cellular and environmental forces, and can also be easily picked up and manipulated by sterile forceps. This allows sponges to be transferred to different wells, or inverted to seed or observe cells on both sides of the sponge.

For a firmer sponge, add Firming Buffer to cells and culture medium prior to adding the cells to the sponge. Alternatively, for cell-culture applications that may be sensitive to free calcium ions, you may rehydrate the sponge with Firming Buffer before adding cells and medium.

AlgiMatrix™ Dissolving Buffer

AlgiMatrix™ Dissolving Buffer (sold separately) is a quick and easy way to recover cells from the AlgiMatrix™ bioscaffold. AlgiMatrix™ Dissolving Buffer is designed to dissolve sponges quickly and completely without disrupting cell spheroids, clusters, or other 3D structures. It is safe to use with all cell types and can be used to dissolve an individual sponge in an AlgiMatrix™ plate well or multiple sponges in a centrifuge tube or other vessel. A protocol for its use is provided on page 4.

Culturing Cells with AlgiMatrix™ Firming Buffer

AlgiMatrix™ Firming Buffer is required for 6-well AlgiMatrix™ plates, and optional but highly recommended for 24-well and 96-well plates. Below is a general protocol for culturing cells using Firming Buffer. Volumes may be optimized for specific cell types. All amounts are given on a per well basis.

Recommended Concentration of Firming Buffer

For general use, we recommend a Firming Buffer concentration of 10% (v/v) in solution. For a firmer AlgiMatrix™ sponge, the concentration can be increased as high as 50% (v/v). Lower Firming Buffer concentrations will lead to softer sponges with better optical clarity. Concentrations lower than 5% (v/v) may result in a sponge that is too soft and prone to damage during medium removal.

Note: For cell-culture applications that may be sensitive to free calcium ions, you may rehydrate the sponge with Firming Buffer **before** adding cells and medium; see the protocol at the bottom of this page.

1. Resuspend the cells in standard cell culture medium, and then add 10% (v/v) AlgiMatrix™ Firming Buffer to this suspension (i.e., 1 part Firming Buffer to 9 parts cells plus medium). The optimal final cell concentration will vary by cell type, but in general 1×10^6 cells/mL is a reliable target. While 10% (v/v) Firming Buffer provides an optimal balance between sponge transparency and firmness, certain applications may benefit from optimizing this concentration.
2. Remove the AlgiMatrix™ 3D Culture System plate from its package and discard the desiccant.
3. Depending on your plate type, inoculate the following volume of the suspension from Step 1 onto the top of each dry sponge with a pipette:

6-well Plate	24-well Plate	96-well Plate
2 mL	400–500 µL	80–120 µL

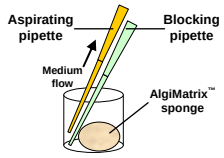
4. **Optional:** Dynamically seed the sponges by immediately centrifuging the plates at 100 × g for 4 minutes. Certain cell types may be embedded more thoroughly within the sponge with dynamic seeding.

Note: The sponge will become wet and translucent. Gas bubbles may appear in the matrix; they will decrease or disappear with time. If large bubbles appear inside or under the sponge, release by gently pushing the sponge against the plate bottom with pipette tip.

5. Approximately 5 minutes after rehydration, the sponge should appear foamy with a dry or soggy surface. Depending on your plate and cell type, add the following additional volumes of culture medium **without** Firming Buffer to the top of each sponge (volumes are intended as a general starting point; volumes may vary by cell type):

6-well Plate	24-well Plate	96-well Plate
2–3 mL	400–500 µL	80–120 µL

6. Incubate the plate(s) in an incubator (36–38°C in a humidified atmosphere of 4–6% CO_2 in air). **Do not stack multiple plates.**
7. Change the medium based on cell proliferation or when the medium begins to change color. To observe the cells inside or on top of the AlgiMatrix™ sponge, decrease the medium volume or relocate the sponge to a dry well. Sponges can be inverted for better observation of cells at the top of the sponges.



Note: Do not allow the aspirating pipette tip to contact the sponge when removing spent medium. Keep the tip on an angle against the wall of the well and block the tip with another pipette tip to avoid sucking up the sponge (see image). If the sponge becomes stuck in the aspirator, stop suction right away and release the sponge, then gently try again. If the sponge has been partially aspirated, do not use that well for quantitative assays. Refer to **Tips** on the first page for additional suggestions regarding medium exchange.

8. After several days, remove the plate from the incubator and examine under light microscopy (low magnification) for the presence of spheroid formation.

Alternative Firming Buffer Protocol for Cell Culture Applications that Require Lower Calcium Levels

AlgiMatrix™ Firming Buffer contains elevated calcium levels. If lower calcium environments are required for specific cell culture applications, modify the first steps of the culturing protocol as follows:

1. Add cell culture medium with 10% (v/v) Firming Buffer but **without** cells to the AlgiMatrix™ sponge.
2. Rehydrate for ~5 minutes.
3. **Optional:** If you are using a high concentration of Firming Buffer in the cell seeding medium (e.g., 50% v/v), rinse the sponge once with culture medium without Firming Buffer.
4. Inoculate the cells in standard culture medium (without Firming Buffer), and then proceed with incubation.

Culturing Cells without Firming Buffer (24-well and 96-well Plates Only)

Though the use of Algimatrix™ Firming Buffer is recommended for all plate types, cells may be cultured without Firming Buffer in 24-well and 96-well plates as described below. Inoculate at low density or high density as indicated below, or optimize for specific cell types.

In general, cells inoculated at low density can be cultured 5 days without medium exchange, while cells inoculated at high density need daily refeeding. All amounts are given on a per well basis.

1. Remove the AlgiMatrix™ 3D Culture System 24-well or 96-well plate from the package. Discard the desiccant.
2. Remove the cells from culture, resuspend in culture medium, and inoculate each sponge with the suspension as follows:

Culture Type	96-well plates	24-well plates
Low-density culture	25,000 cells/well: Resuspend at a concentration of 8.33×10^5 cells/mL. Inoculate 30 µL into the middle of each dry sponge with an electronic 8-channel multichannel pipette.	125,000 cells/well: Resuspend at a concentration of 0.417×10^6 cells/mL. Inoculate 300 µL into the middle of each dry sponge with a pipette.
High-density culture	300,000 cells/well: Resuspend at a concentration of 1×10^7 cells/mL. Inoculate 30 µL into the middle of each dry sponge with an electronic 8-channel multichannel pipette.	1.5 million cells/well: Resuspend at a concentration of 5.0×10^6 cells/mL. Inoculate 300 µL into the middle of each dry sponge with a pipette.

3. **Optional:** Dynamically seed the sponges by immediately centrifuging the plates at 100 × g for 4 minutes. Certain cell types may be embedded more thoroughly within the sponge with dynamic seeding.
4. Place the plate in an incubator (36–38°C in a humidified atmosphere of 4–6% CO_2 in air) for 10 minutes. **Do not stack multiple plates.**
5. Remove the plate from the incubator and place in a hood. Add the following volume of cell-culture medium at room temperature to each well:

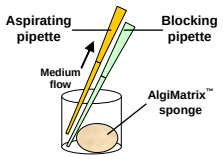
24-well Plate	96-well Plate
1000 µL	200 µL

Note: Immediately after inoculation, numerous air bubbles will be present in the scaffold. This is normal; the bubbles will disappear after 2–3 days in inoculated wells as the cells consume oxygen.

6. Incubate plate(s) in an incubator (36–38°C in a humidified atmosphere of 4–6% CO_2 in air). To avoid edge-effect evaporation of water from the outer wells (a problem with some incubators), place plate(s) on a moistened paper towel in a container covered with perforated aluminum-foil.
7. In general, low-density cultures can go 5 days without medium exchange. For high-density cultures, replenish medium daily by gently withdrawing the following volume of spent medium and replacing it with an equivalent amount of fresh medium:

24-well Plate	96-well Plate
250–750 µL	50–150 µL

Note: Cultures inoculated at lower densities may need media replacement when media turns yellow.



Note: Do not allow the pipette tip to contact the bioscaffold when removing spent medium. Keep the tip on an angle against the wall of the well to avoid sucking up the sponge (see image to the left). If loose bioscaffolds interfere with media refeeding, add medium to each well (~100 µL for 96-well plates, ~500 µL for 24-well plates). Centrifuge the plates at 400 × g for 7 minutes, then remove medium and add fresh medium, repeating once if desired.

8. After several days, remove the plate from the incubator and examine under light microscopy (low magnification) for presence of spheroid formation.

Measuring Cell Viability

- To assess cell viability and proliferation, use alamarBlue® directly on spheroids within the AlgiMatrix™ 3D Culture System according to the manufacturer's instructions.
- Viability can be visually assessed by using the LIVE/DEAD® Viability/ Cytotoxicity Kit. For optimal visualization, first dissolve the sponges using AlgiMatrix™ Dissolving Buffer and then fluorescently label the cells.
- For Trypan Blue viability determination, dissolve sponges and thoroughly wash cells prior to addition of Trypan Blue solution. Failure to wash cells will result in a high background reading. Do not use automated cell counting with this method.

Antibody Staining

For antibody staining, embed AlgiMatrix™ 3D Culture System-containing spheroids in paraffin according to your regular protocol before sectioning.

Using AlgiMatrix™ Dissolving Buffer

The amount of AlgiMatrix™ Dissolving Buffer required to dissolve a sponge depends on the size of the AlgiMatrix™ plate well and the concentration of AlgiMatrix™ Firming Buffer (if any) used on the sponge. Use the following table to determine the volume of Dissolving Buffer to use per AlgiMatrix™ plate well.

AlgiMatrix™ Plate Well Format	Volume of Dissolving Buffer per Well If the Sponge Contains…				
	0–10% Firming Buffer	20% Firming Buffer	30% Firming Buffer	40% Firming Buffer	50% Firming Buffer
6-Well Plate	5 mL	7.5 mL	10 mL	12.5 mL	15 mL
24-Well Plate	1 mL	1.5 mL	2 mL	3 mL	4 mL
96-Well Plate	0.1 mL	0.15 mL	0.2 mL	0.3 mL	0.4 mL

1. Using a pipette, aspirate all the residual liquid media from the vessel containing the sponge, leaving the wet sponge only.
2. Use the table above to determine the volume of Dissolving Buffer to add per well. Add that volume directly onto the sponge.

Note: Alternatively, you can transfer one or more AlgiMatrix™ sponges to a separate vessel (e.g., a centrifuge tube) for dissolving. In this case, use enough Dissolving Buffer to fully submerge the sponge(s). Cell culture medium can be added to the suspension if desired (0.25 mL per mL of Dissolving Buffer).

3. Incubate for ~5 minutes, while examining the sponge under a microscope. Typically, the sponge begins to visibly degrade within 5 minutes after adding Dissolving Buffer. If no cell release is observed within 5 minutes, add another volume of Dissolving Buffer directly onto the sponge (per the table above) and incubate for an additional 5 minutes. After 10 minutes, the sponge should be completely degraded.

Note: We recommend leaving cells in Dissolving Buffer for no longer than 30 minutes.

4. Gently aspirate the contents of the well and transfer to a sterile centrifuge tube or multiple tubes. Add culture medium to the centrifuge tube(s).
5. Centrifuge at 200 × g for 5 minutes to pellet the released cells, then remove the supernatant and resuspend the pellet as desired.
6. **Optional:** To remove any debris that remains with the recovered cells, add an equivalent volume of Dissolving Buffer to the cell suspension and gently agitate for up to 2 minutes. Repeat the centrifugation procedure as above.

Isolating Individual Cells from Spheroids

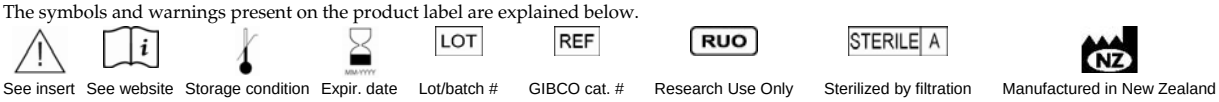
1. To isolate individual cells, first isolate spheroids using Dissolving Buffer as described above.
2. Add 5 mL of TrypLE™ Select or Trypsin-EDTA to the centrifuge tube with spheroids, place at 37°C, and triturate over 5–10 minutes until spheroids are dissociated.
3. After dissociation of spheroids, add 5 mL of growth medium, spin 7 minutes at 400 × g, and remove supernatant. Then proceed with your assay.

Related Products

Product	Size	Catalog no.	Product	Size	Catalog no.
Phosphate-buffered saline (PBS) 7.2 (1X), liquid	500 mL	20012	KNOCKOUT™ Serum Replacement	500 mL	10828
Phosphate-buffered saline (PBS) 7.4 (1X), liquid	500 mL	10010	Defined Keratinocyte-SFM (1X), liquid	500 mL	10744
Dulbecco's Phosphate-Buffered Saline (DPBS) (10X), liquid, without calcium or magnesium	500 mL	14200	LIVE/DEAD® Viability/ Cytotoxicity Kit	1 kit	L-3224
TrypLE™ Select (1X), liquid, without Phenol Red	100 mL	12563	Human Endothelial-SFM (1X), liquid	500 mL	11111
Trypsin, 0.05% (1X) with EDTA	100 mL	25300	Hepatozyme-SFM (1X), liquid	500 mL	17705
alamarBlue®	25 mL	DAL1025	Versene 1:5,000 (1X), liquid	100 mL	15040

Explanation of Symbols and Warnings

The symbols and warnings present on the product label are explained below.



See insert | See website | Storage condition | Expir. date | Lot/batch # | GIBCO cat. # | Research Use Only | Sterilized by filtration | Manufactured in New Zealand

Purchaser Notification

This product is covered by Limited Use Label License No. 5, Invitrogen Technology (for details, visit our website at www.invitrogen.com). By the use of this product you accept the terms and conditions of this Limited Use Label License.

Technical Support

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at www.invitrogen.com. For further assistance, please email our Technical Support team at techsupport@invitrogen.com.