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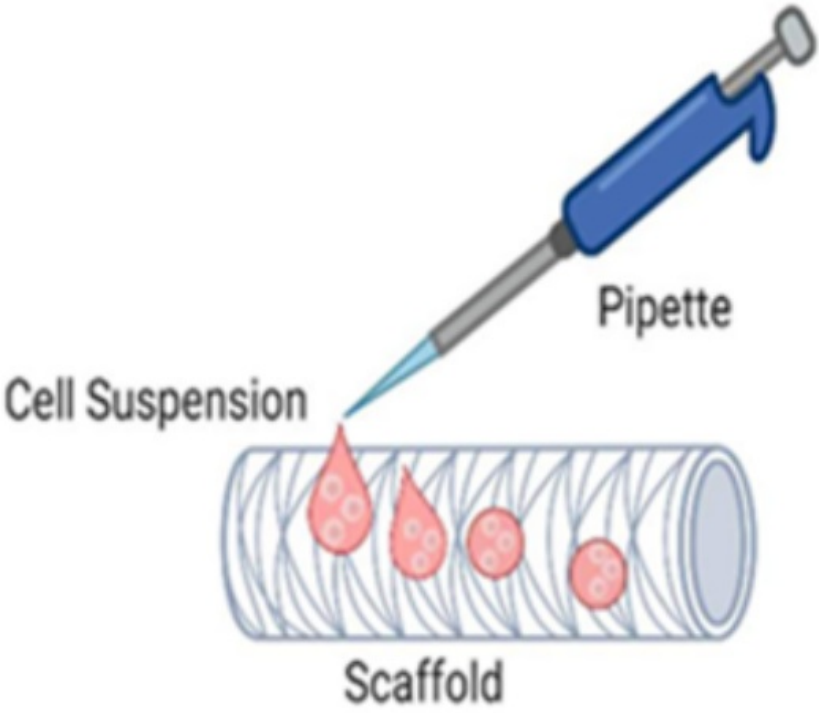
VivoTex Scaffolds Suspended Cells Seeding



Scaffold Designs: Box, Aligned, and Isotropic Patterns

Intended use

VivoTexTM scaffolds are custom-designed to augments your research, enhancing results that advance your scientific discoveries. VivoTexTM scaffolds are shipped non-sterile, placed inside low-attachment tissue culture plates, and can be used directly for cell culture applications. The scaffold kit does not contain any living cells or materials. VivoTexTM scaffolds are suited for research use only; they are not intended for clinical or household use.

Content and storage

Catalog number	Content	Storage
VBX.0001.24.24.S	24x **Box Pattern** MEW Scaffolds 10 µm fibers, 150µm spacing, 200µm thick, PCL, 24 Well, 24 Scaffolds (non-sterile)	Store at cool, dry, well-ventilated area.
	1x 24-well plate, Ultra Low-Attachment (non-sterile)	
VAL.0001.24.24.S	24x **Aligned Pattern** MEW Scaffolds 10 µm fibers, 100µm spacing, 200µm thick, PCL, 24 Well, 24 Scaffolds (non-sterile)	Store at cool, dry, well-ventilated area.
	1x 24-well plate, Ultra Low-Attachment (non-sterile)	
VIS.0001.24.24.S	24x **Isotropic Pattern** MEW Scaffolds 10 µm fibers, 150µm spacing, 200µm thick, PCL, 24 Well, 24 Scaffolds (non-sterile)	Store at cool, dry, well-ventilated area.
	1x 24-well plate, Ultra Low-Attachment (non-sterile)	

See kit label for expiration dates. See bottle labels for expiry dates of individual components.

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OVERVIEW

Required materials and equipment (not supplied)

- Cell suspension: Prepare cells in suspension at a recommended concentration of 1 × 10^6 cells/mL in culture medium.
- Culture Medium: It is recommended to use cell culture medium supplemented with 10% (v/v) serum (e.g. Fetal Bovine Serum) and 1% (v/v) antimicrobial agents (e.g., Penicillin-Streptomycin).
- Water bath or heat block
- Sterile Pipettes and pipette tips
- Biosafety cabinet
- Incubator (37°C, 5% CO_2)
- Ethanol for work area sterilization
- Sterile forceps (optional for scaffold handling)
- Sterile 1.5 mL microcentrifuge tubes (optional for scaffold handling)

Procedural guidelines

- All steps should be performed using biological aseptic techniques using a Biological Safety cabinet to maintain sterility of the product.
- All kit components can be used at room temperature (18-25°C)
- Use disposable, individually wrapped, sterile plasticware and sterile tubes, pipettes tips, and forceps.
- The biological performance is dependent on the cells used. It is recommended to always include a 2D culture control (without VivoTexTM scaffolds) to confirm cell attachment capacity using cell-culture treated well plates.
- Wear appropriate Personal Protective Equipment in accordance with the biological hazards introduced in your culture protocol (cell type and culture medium)

PREPARATION

Sterilization of non-sterile VivoTexTM MEW scaffolds

- One day before starting your experiment, immerse VivoTexTM MEW scaffolds in 70% ethanol for 30 minutes under sterile conditions.
- Wash the scaffolds with Phosphate Buffer Solution (Solution B) three times (5min per wash) under sterile conditions.

Pre-treatment 1: Etching VivoTexTM Scaffolds

- One day before of your experiment, treat the VivoTexTM scaffolds with the supplied etching solution (solution A) for 10-30 minutes under sterile conditions.
- Wash the scaffolds with Phosphate Buffer Solution (Solution B) three times (5min per wash) under sterile conditions.
- Remove solution B from the scaffolds and allow the scaffolds to air dry inside the biosafety cabinet, leaving the lid off the well plate (1-2h).
- Proceed with the steps for protein adsorption or cell seeding, as outlined below.

Pre-treatment 2: Protein adsorption of VivoTexTM Scaffolds

- Before starting your experiment, treat the VivoTexTM scaffolds with serum-containing media (e.g. 10% FBS) for 2-24 hours under sterile conditions.
- Remove the culture media and proceed with the steps for seeding cells outlined below.
- Remove solution B from the scaffolds and allow the scaffolds to air dry inside the biosafety cabinet, leaving the lid off the well plate (1-2h).

PROTOCOL

Seeding of cells onto VivoTexTM MEW Scaffolds

1. Prepare a cell suspension at 1×10^6 cells/mL.
2. Carefully pipette 100 µL of cell suspension (containing 100,000 cells) directly onto the center of each VivoTexTM MEW Scaffolds.
3. Allow cells to attach for 1-3 hours at 37°C in the incubator without additional media. Note: Do not let the samples dry out. As needed, carefully replenish with 10-40 µL of

cell culture medium ~each hour without overflowing the scaffold or dissociating the cells from the sample. The incubation time is dependent on the cell type used. Include a 2D control to confirm cell attachment of the specific cell type used. Confirm initial seeding and attachment under an inverted microscope before adding more media.

4. After incubation, gently add 900 µL of cell culture medium to each well to bring the total volume to 1 mL per well.

Culturing VivoTexTM MEW Scaffolds

1. Incubate the well plate with the cell-seeded samples at 37°C and 5% CO_2.
   Note: The scaffolds are compatible with a wide range of standard CO_2 incubators, tri-gas incubators, and hypoxia chambers designed for cell culture applications. Select the appropriate incubation environment based on the experimental requirements.
2. Replace the culture media according to your standard cell culture protocol (e.g., every 2–3 days).
   - Carefully aspirate the spent media from the side of each well to avoid disturbing the scaffolds.
   - Gently add 1 mL of pre-warmed complete DMEM to each well.
     Note: Ensure all media handling is performed under sterile conditions to maintain culture integrity.
3. Determine culture duration based on the intended downstream application:
   - Short-term culture (24–72 hours): Recommended for studies focused on cell attachment and proliferation.
   - Long-term culture (4–56 days): Suitable for applications involving extracellular matrix production or cellular differentiation.

Note: Optimal culture duration may vary depending on cell type and experimental design. Always refer to your specific assay requirements

TROUBLESHOOTING

- If scaffold flotation is observed during seeding or early culture, consider the following approaches to promote stability:
  - Pre-wet the scaffolds with complete culture media for an extended period prior to cell seeding to enhance saturation and reduce buoyancy.

    - Use sterile weighting devices, such as stainless steel or Teflon rings, to gently anchor the scaffolds without compromising sterility or cell viability.
      Note: Ensure that any materials used for weighting are biocompatible and have been sterilized according to your laboratory's protocols.
- To promote uniform cell distribution across the scaffold, minimize movement of the culture vessel during the first few hours post-seeding. Furthermore, avoid adding excess culture media too early, as this may dislodge cells from the scaffold surface, particularly at the edges. Smaller media volumes can be used by preparing a more concentrated cell suspension (1-10 × 10^6 cells/mL) toensure a droplet based seeding method.
- If poor cell attachment is observed, verify the incubation time required for initial cell adhesion by performing a comparative optimization using standard 2D tissue culture conditions. Note: Scaffolds typically require longer incubation times for effective cell attachment compared to standard tissue culture plastic controls.

FAQ

- **Q: Can VivoTexTM scaffolds be used for clinical applications?**
    - A: No, VivoTexTM scaffolds are intended for research use only and are not suitable for clinical applications.
- **Q: How should I store the VivoTexTM scaffolds?**
    - A: Store the scaffolds in a cool, dry, and well-ventilated area. Check the kit label for expiration dates.

Documents / Resources

	VivoTex Scaffolds Suspended Cells Seeding [pdf] User Guide VBX.0001.24.24.S, VAL.0001.24.24.S, VIS.0001.24.24.S, Scaffolds Suspended Cells Seeding, Scaffolds, Suspended Cells Seeding, Cells Seeding , Seeding

References

- User Manual

VivoTex

Cells Seeding, Scaffolds, Scaffolds Suspended Cells Seeding, Seeding, Suspended Cells Seeding, VAL.0001.24.24.S, VBX.0001.24.24.S, VIS.0001.24.24.S, VivoTex

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