VivoTex™ Microfiber Scaffolds for Suspended Cells Seeding

Instructions For Use / Users Guide

Scaffold Designs: Lattice, Axis, and IsoMesh

Intended use: VivoTex™ scaffolds are custom-designed to augments your research, enhancing results that advance your scientific discoveries. VivoTex™ scaffolds are shipped 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. VivoTex™ scaffolds are suited for research use only. Not approved for applications to humans or animals, or for diagnostic use.

Storage: Room temperature with dry, well-ventilated area, and protected from sunlight.
See kit label for expiration dates. See bottle labels for expiry dates of individual components

Required materials & 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 (BSC)
- 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. It is recommended to include a 2D monolayer culture control (without VivoTex™ 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)

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VivoTex™ microfiber scaffold preparation

1. Pre-treatment (option): Protein adsorption of VivoTex™ Scaffolds
    - Treat the scaffolds with serum containing media (e.g. 10% FBS) for 24 hours under sterile conditions.
    - Remove the culture media and proceed with the steps for seeding cells outlined below.

Seeding cells onto VivoTex™ Scaffolds

1. Prepare cell-hydrogel suspension for 1 × 10^6 cells/mL.
    - Example workflow - Matrigel:
        1. Thaw Matrigel aliquot at 4°C overnight before experiment
        2. Lift cells from expansion flasks, count them, and centrifuge
        3. Dilute Matrigel to working solution ~ 1.0-2.0 mg/mL
        4. Resuspend cells in Matrigel solution
2. Pipette 100 µL of cell suspension (containing 100,000 cells) directly onto the center of each Scaffold.
3. Allow solution to gel for 30 min at 37°C in the incubator without adding 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 VivoTex™ MEW Scaffolds

1. Incubate the well plate with the cell-seeded samples at 37°C and 5% CO_2.

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) to ensure 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.