BioSurfaces
Nanomaterial Solutions

6-Well (IIC6), 12-Well (IIC12) and 24-Well (IIC24) Individual Inserts with Bio-Spun™ Scaffolds User's Guide

Product Information

Bio-Spun™ In Vitro Research Tools (IVRT) are convenient, sterile, easy-to-use permeable support devices for the study of cellular proliferation, dose-responses, transport and other metabolic activities *in vitro*. The 3D nanofibrous Bio-Spun™ scaffolds are uniquely designed to replicate the body's natural extracellular matrix both in structure and scale, thereby eliminating the need for additional animal derived products and their associated drawbacks – xenogeneic sources, undefined components, and batch-to-batch variability- commonly used in the preparation of 3D cell cultures. Bio-Spun™ scaffolds are mounted onto various size Corning Transwell® individual inserts (6-well, 12-well or 24-well formats) (example in Figure 1) and are available with different polymers comprising the scaffold: polyester (PET), polyurethane (PU), poly (D,L-lactide-co-glycolide)(PDLGA) or PDLGA/poly-l-lactic acid bilayer (PDLGA/PLLA).

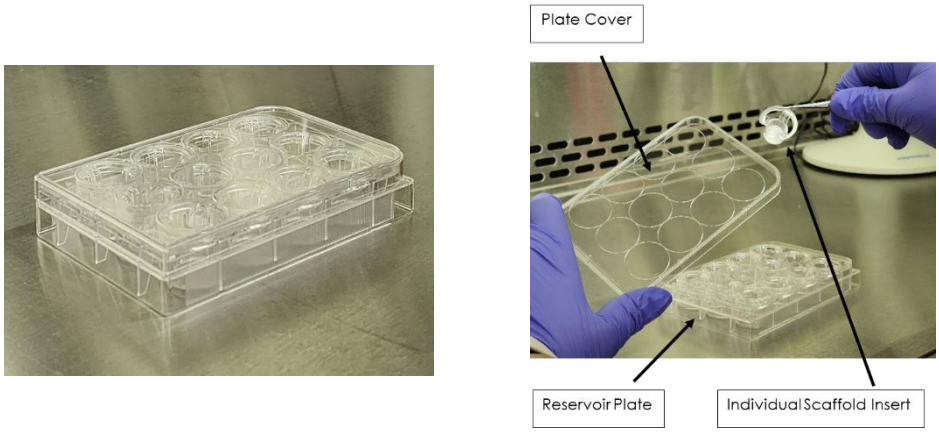

Figure 1: Picture of an individual scaffold plate assembly, showing assembled 12-well plate (left panel) and individual reservoir plate, individual scaffold inserts and cover components (right panel).

General Directions for Using Bio-Spun™ individual inserts

(Note: All Steps Outlined Below Must Be Done with Sterile Solutions/Materials Using Sterile Technique in a Tissue Culture Hood)

1. Bio-Spun™ individual inserts are used by first adding medium to the bottom reservoir plate, followed by adding the individual inserts to the plate, and lastly adding the medium and cells to the inside compartment.

2. To remove media, aspirate from the bottom reservoir plate first and then from the inside (apical side) of the individual insert. To feed, add media to the insert first and then to the bottom reservoir plate.
3. To improve cell attachment, the Bio-Spun™ scaffolds should be pre-wet before growing the cells. Please see Table 1 below for pre-wetting instructions. **Process for pre-wetting should be performed under sterile conditions.** The sequence of media removal/addition is always the same. Incubation temperature is the same temperature that will be used to grow the cells. Recommended medium volumes to use inside the well (apical side) and in the reservoir plate are shown in Table 2. **Care should always be used to not puncture or damage scaffold using aspirator or pipettor tip.**

Table 1. Pre-wetting Bio-Spun™ Individual Inserts

Scaffold material	Pre-Wetting Step 1	Pre-Wetting Step 2	Pre-Wetting Step 3
PU	Add a 20% ethanol solution (in sterile water) to both the inserts and the reservoir plate and incubate for 60 minutes at 37°C in an incubator.	Aspirate out the ethanol solution, and add sterile water to both the inserts and the reservoir plate and incubate for 60 minutes at 37°C in an incubator.	Carefully aspirate out the water and add desired cell culture media (with or without serum proteins) and incubate for at least 60 minutes at 37°C.
PET	Add a 20% ethanol solution (in sterile water) to both the inserts and the reservoir plate and incubate for at least 60 minutes at 37°C in an incubator.	Aspirate out the ethanol solution, and add sterile water to both the inserts and the reservoir plate and incubate for at least 60 minutes in an incubator.	Aspirate out the water and add cell culture media (with or without serum proteins) and incubate for at least 60 minutes at 37°C.
PDLGA or PDLGA/PLLA Bilayer	**Do not ever use ethanol with PDLGA or PDLGA/PLLA Bilayer Scaffolds**	N/A	Add cell culture media (with or without serum proteins) and incubate for at least 60 minutes (overnight preferred) at 37°C.

4. Following pre-wetting, aspirate out media from the reservoir plate and the inserts. With the insert in place, add fresh medium to the reservoir plate. Then add cells in fresh medium to the insert and return to the incubator.

For more information, please contact us
via e-mail (info@biosurfaces.us) or by phone (1-508-881-8860)

5. Cells growing on the Bio-Spun™ scaffolds may be fixed and stained while in the insert plate using standard histology techniques. Avoid using solvents that dissolve polyester membrane materials, PU, PDLGA or PLLA. Further processing steps may be carried out by detaching the scaffolds by making incisions with a sterile surgical scalpel following the circumference of the plastic chamber.

Table 2. Recommended Medium Volumes for Bio-Spun™ Individual Inserts Per Corning Instructions*

Individual Insert Type (Well Diameter in mm)	Recommended Volume for Bio-Spun™ Scaffold (Apical/Top Side)	Recommended Volume for Reservoir Well
24-well (6.5 mm)	0.1 mL	0.6 mL
12-Well (12 mm)	0.5 mL	1.5 mL
6-Well (24 mm)	1.5 mL	2.6 mL

*https://www.corning.com/catalog/cls/documents/protocols/Transwell_InstructionManual.pdf

Helpful Hints:

1. Cells grown on the Bio-Spun™ scaffolds are often sensitive to initial seeding density for good cell attachment. When using these scaffolds for the first time, it is advisable to experiment with a range of seeding densities to achieve optimum growth results.
2. Use care when transporting the plates to the incubator after cell seeding. Avoid spilling medium from the individual insert into the bottom reservoir plate, which will carry suspended cells into the reservoir plate.
3. The frequency of medium changes and media volumes will vary based on the specific cell type and the duration of the culture. If your medium contains a pH indicator, use its color as a as a reference to determine the appropriate timing for medium changes.
4. The success of assays relying on cell adhesion to the Bio-Spun™ scaffolds requires a precise order of media removal and replacement. When feeding the plates, it is crucial to maintain net positive hydrostatic pressure above the cells to prevent their detachment from the scaffolds. Therefore, whether employing manual mode or with automation, the sequence of media removal/addition is always the same.

For more information, please contact us
via e-mail (info@biosurfaces.us) or by phone (1-508-881-8860)

5. To avoid damaging the cell culture surface, take care not to allow pipette tips to come in contact with the Bio-Spun™ scaffolds when removing or adding cells or medium to the plates. Use caution when removing medium via suction as scaffold can be damaged.

For more information, please contact us
via e-mail (info@biosurfaces.us) or by phone (1-508-881-8860)