

Trial Seeding Protocol

3D Biotek recommends following this Trial Seeding Protocol if you are using 3D Insert™ scaffolds for the first time. This small volume protocol will ensure that your cells are seeded directly onto the center of the scaffold with minimal chances for cells to contact the sides of wells and/or drop down to the bottom of the well.

Materials and Equipment

Sterile ***non-treated*** cell culture plate
Sterile forceps
Pipette
Sterile pipette tips
Vacuum
Biosafety hood
Cell culture incubator

Procedure

1. Remove cells of interest from culture and resuspend in culture medium at an appropriate concentration.
2. Slowly pipette the correct volume (refer to chart below) of this cell suspension onto the center, top surface of each 3D Insert™ scaffold. **Note: Do not allow the cell suspension to contact the sides of the wells. This will result in reduced seeding efficiency.**

Scaffold Size	Normal Seeding Volume	TRIAL SEEDING VOLUME*
96-well scaffold	20 µl	10 µl
48-well scaffold	50 µl	25 µl
24-well scaffold	100 µl	50 µl
12-well scaffold	200 µl	100 µl

* Please note that TRIAL SEEDING VOLUMES represent 50% of the normal cell seeding volumes. Depending on your preference or experience, these can be adjusted to 70-80% of normal cell seeding volumes.

3. Gently place plates into incubator. Avoid agitating the plates!
4. After 3 h, remove the plates containing the scaffolds from the incubator. Add the appropriate volume (see chart below) of fresh medium into each well in a sterile biosafety hood. Make sure that the scaffolds are completely immersed and sitting at the bottom of the wells. Return the plate to the incubator.

Scaffold Size	Volume to Add After 3 h	Total Volume After 3 h
96-well scaffold	190 µl	200 µl
48-well scaffold	225 µl	250 µl
24-well scaffold	450 µl	500 µl
12-well scaffold	1400 µl	1500 µl

5. After 24 h, carefully aspirate the medium around the scaffolds, and then transfer the scaffolds into new wells with sterile forceps. Add fresh medium into each new well. Again, make sure that the scaffolds are sitting at the bottom of the wells. Continue with normal cell culture until analysis.