GRADIENTECH

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Protocol for Loading Hypoxia-Sensitive Biological Samples in CellDirector® 3D

For example: Neurons

Degassing your CellDirector before loading the chamber with sample/3D matrix is important to minimise the risk of air bubble growth within the matrix during the experiment. Air bubbles within the matrix can affect the gradient formation, as well as the microenvironment of the cells.

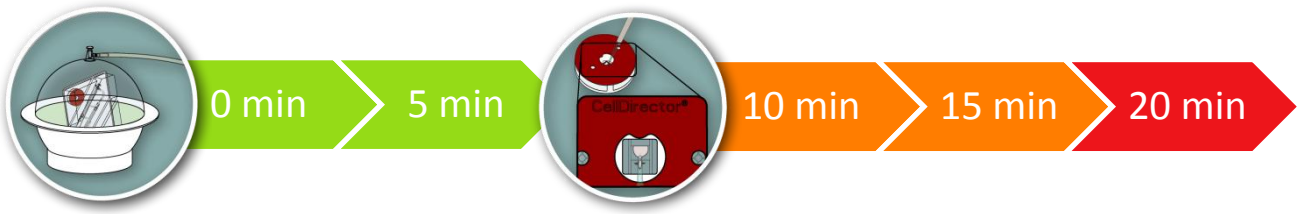
By degassing your CellDirector, the microbubbles initially present within the 3D matrix after sample/3D matrix loading are eliminated due to gas equilibration of the surrounding assay.

Note that when working with hypoxia-sensitive biological samples, such as some types of neurons, the cells can be negatively affected by the lower oxygen concentration in CellDirector that occurs immediately after degassing. It is therefore recommended that loading of hypoxia-sensitive material is somewhat delayed after the degassing step.

Follow the protocol below to avoid air bubble formation when working with hypoxia-sensitive biological material.

1. Place CellDirector 3D for 30 min in a degassing chamber*
2. *For cells and tissues not sensitive to hypoxia:*
   Load the matrix/sample within 5 min for most efficient air bubble removal in the matrix. Load 8 µl matrix/sample.

   For hypoxia-sensitive biological material:
   Wait 5 min after degassing CellDirector 3D. Thereafter load 8 µl matrix/sample within the next 10 min.
3. Make sure that the matrix has polymerised before proceeding (see Short User Guide).



* equilibrate the assay to the same temperature as the matrix to minimise air bubbles, i.e. degass CellDirector on ice when using Matrigel™ matrix