One-Week Training Schedule

Focus: Fundamental technologies, workflows, and instrument handling for 36PillarPlate and 36PerfusionPlate

Objective: This training will provide a practical, technology-focused introduction to the 36PillarPlate and 36PerfusionPlate systems. Participants will learn essential concepts such as pillar/perfusion plate geometry, hydrogel handling and crosslinking, static and dynamic 3D cell cultures, digital rocker setting, and the proper use of accessories such as the LoadingPlate and PetriLid. Hands-on sessions will emphasize accurate cell loading on the pillar plate, plate alignment and assembly, perfusion operation, culture medium change, and basic assay and imaging workflows.

Day 1 (Monday)

Introduction & System Overview

Goal: Build foundational understanding of the platform and its intended applications.

Materials required: 36PillarPlate, 36PerfusionPlate, 384DeepWellPlate, LoadingPlate, PetriLid, ULA 384-well plate

Lecture + Demo

- Overview of pillar/perfusion plate technology
  - Pillar geometry, perfusion flow mechanics, hydrogel compatibility
  - Static vs. dynamic culture concepts
- Overview of accessories: LoadingPlate, PetriLid
- Key applications:
  - 3D cell loading, encapsulation, viability assays, imaging workflows

Hands-On

- Plate handling: 36PillarPlate, 36PerfusionPlate
- Demonstration: Plate assembly/disassembly

Day 2 (Tuesday)

Hydrogel Preparation and Handling & Manual Loading of Hydrogel/Cell Suspension

Goal: Training on foundational skills for loading hydrogels alone and cell suspension in hydrogels onto the pillar plate.

Materials required: Matrigel, alginate, gelatin, Hep3B cells, 36PillarPlate, 1 mL pipette and pipette tips, LoadingPlate, 36PetriLid, petri dish

Lecture + Demo

- Introduction to hydrogels used in the workflow:
  - Matrigel, alginate-gelatin (properties, viscosities, temperature sensitivity)
- Preparation of Matrigel and alginate
- Best practices for manual dispensing with a 1 mL pipette and cell loading with a LoadingPlate (pipette types, volumes, timing, avoiding bubbles)

Hands-On

- Loading Matrigel alone and cell suspension in Matrigel on pillars: (1) dispensing with a 1 mL pipette and (2) loading with a LoadingPlate
- Loading alginate-gelatin alone and cell suspension in alginate-gelatin on pillars: (1) dispensing with a 1 mL pipette and (2) loading with a LoadingPlate
- Users practice:
  - Manual loading of alginate-gelatin and cell suspension in alginate-gelatin onto the 36PillarPlate with a 1 mL pipette and a LoadingPlate
  - Temperature management and gelation control

- Imaging:
    - How to image single cells on the pillar plate
    - Focusing, Z-stacking, field-of-view considerations
- Troubleshooting session:
    - Air bubble (micro and macro bubbles) avoidance
    - Uneven loading of cells and hydrogels
    - Prevention of spot detachment

Day 3 (Wednesday)

Spheroid Formation, Transfer to Pillar Plate, and Culture

Goal: Training on experimental workflows for spheroid formation, transfer, and culture on the 36PillarPlate.

Materials required: Hep3B cells, cell culture medium, ultralow attachment (ULA) 384-well plate, 36PillarPlate, 384DeepWellPlate, LoadingPlate, 36PetriLid, petri dish

Lecture + Demo

- Overview:
    - Spheroid formation and transfer
    - Optimization of cell density for spheroid formation in ULA 384-well plates, hydrogel viscosity, and optimum media volume
    - Troubleshooting common spheroid transfer errors
- Demonstration of standard spheroid transfer workflow

Hands-On

- Practice spheroid formation and transfer to the pillar plate
- Imaging:
    - How to image spheroids on the pillar plate
    - Focusing, Z-stacking, field-of-view considerations
- Viability assay demo:
    - Calcein AM/ethidium homodimer-1 assay and CellTiter-Glo assay
- Troubleshooting session:
    - Unsuccessful spheroid transfer
    - Spheroids located out of pillar center
    - Rapid 2D growth

Day 4 (Thursday)

Perfusion Culture Operation

Goal: Train users how to run dynamic 3D cell culture, assemble the full perfusion plate stack, and understand bidirectional perfusion flow principles.

Materials required: 36PillarPlate loaded with cells/spheroids, 36PerfusionPlate, culture medium, digital rocker from Mimetas

Lecture + Demo

- Perfusion mechanism explanation:
    - Tilt-based perfusion
    - Flow rate vs. tilt angle
    - Media exchange mechanics
- Introduction to the Perfusion Plate setup on the digital rocker

Hands-On

- Full assembly practice: PillarPlate + PerfusionPlate + Digital rocker
- Perfusion operation with a digital rocker: setting tilting angle and frequency
- Running a mock dynamic culture for 1 - 2 hours
- Changing cell culture media
- Troubleshooting session:

- Overflow of cell culture media from the Perfusion Plate

Day 5 (Friday)

Downstream Cell-Based Assays

Goal: Provide operation-level training on cell viability assays and downstream workflows used with pillar/perfusion plates.

Materials required: 36PillarPlate with cells/spheroids, 384DeepWellPlate, assay reagents

Lecture + Demo

- Technical overview of compatible assays:
  - ATP assays (CellTiter-Glo)
  - Fluorescence-based, high-content imaging assays
  - Immunofluorescence (IF) staining of spheroids/organoids
  - RT-PCR assay

Hands-On

- Viability assay, brief demonstration of IF staining, and spheroid detachment
- Data quality considerations:
  - Signal normalization
  - Edge effects
  - Replicate consistency
- How to design plate layouts
- Troubleshooting session:
  - Optimum staining/rinsing time
  - Avoiding photobleaching
  - Efficient detachment of spheroids from the pillars
  - Tissue/organoid clearing for imaging

Day 6 (Saturday)

Workflow Review, Troubleshooting, and Q & A

Goal: Reinforce learning through full workflow execution.

Q & A

- Troubleshooting masterclass:
  - Common loading errors
  - Perfusion setup issues
  - Imaging artifacts
  - Gel integrity problems
- Questions for specific applications