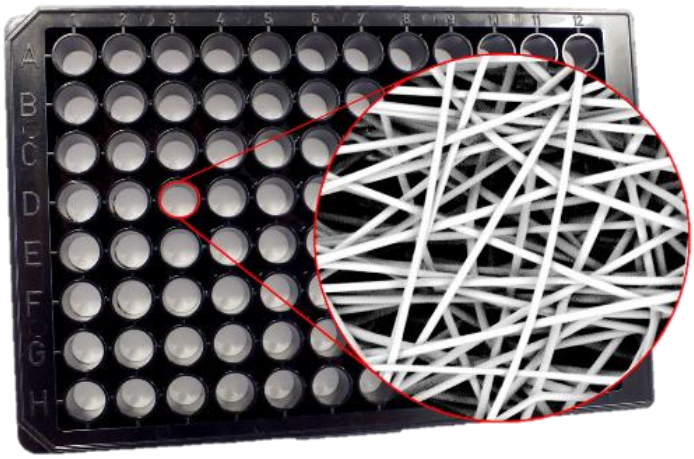
MIMETIX
CELLS IN 3D

Mimetix® Plates Product Manual



The Mimetix 96-well plate is a highly-consistent and easy-to-use 3D cell culture platform. It holds great promise to reduce the number of costly drug failures in clinical trials, by enabling more realistic tumour and toxicology models *in vitro.*

The Mimetix scaffold offers a true 3D micro-environment

ELECTROSPINNING COMPANY

Please visit our website for more information on our products, protocols and to order samples.

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Product description

Mimetix scaffolds mimic the extracellular matrix by providing an ideal architectural environment to support the growth of cells in 3D. They are created by electrospinning the medical-grade polymer poly(L-lactide) (PLLA) into microfibers, which are highly consistent with regard to fibre diameter and pore size, resulting in excellent reproducibility of cell-based assays.

The Mimetix scaffold is incorporated into standard SBS footprint well plate frames (96- and 384) with bases of superior optical clarity and minimal base distortion. The scaffold depth of 50 µm is thick enough to provide the benefits of 3D cell morphology and behaviour, yet thin enough to allow microscopic imaging.

General Features

- True 3D micro-environment
- Minimal protocol adaption required to switch from 2D to 3D
- Compatible with industry-standard automated handling and imaging equipment
- High well-to-well and batch-to-batch consistency

Scaffold specifications

- Material: medical-grade poly-L-lactide (PLLA), FDA-approved
- Scaffold thickness: 50 µm
- Available fibre diameters: 4 µm (= pores of 15-30 µm)
- Overall porosity: app. 80%
- Non-biodegradable in *in vitro* applications
- Supplied with low profile lid with condensation rings

Usage & Handling

- Compatible with fluorescent and light microscopy
- Supplied gamma-irradiated in individually-sealed plastic wrapping
- Scaffolds can be coated with materials to facilitate cell adhesion in low serum conditions

Other available formats:

- 12-well plate with removable scaffold discs
- 6-well plate with hanging inserts
- 12-well plate with aligned scaffold inserts

Instructions for seeding cells into the Mimetix scaffold

The Mimetix scaffold needs to be pre-treated with ethanol in order to allow a cell suspension to access the pores.

For best results follow these steps:

- Add 100 µl of 20% ethanol to each well and allow it to soak into the membrane for 5 minutes, this will wet the scaffold evenly.
- Carefully aspirate ethanol from the edge of the well, avoid touching the scaffold
- Wash the scaffold twice with 150 µl of PBS.
- Leave the scaffold in 100 µl of media until you are ready to seed the cells.
- We recommend seeding 10,000 cells suspended in 100 µl medium per well as a general guideline; or alternatively, whichever cell number the experiment to be conducted requires.
- Culture the cells in the same way as for 2D.

For long-term experiments, we recommend to exchange or semi-exchange the medium every 3 days.

When seeding cells in proliferation assays to determine the IC_{50} of a drug, we recommend you grow the cells in the Mimetix scaffold for 21 days prior to adding the drugs so that the cells are well established in the 3D conditions. Semi exchange the media twice a week during this pre-culture time.

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1 Precondition

The Mimetix scaffold needs to be wetted with ethanol in order to allow a cell suspension to access the pores.

- 96-well plate: Add 100 µL 20% ethanol per well.
- 6- & 12-well plate: Add 1 mL 20% ethanol per well.
- Allow ethanol to soak into the membrane for 5 min, then aspirate ethanol carefully without touching the scaffold.

2 Wash

- Wash scaffold twice with PBS.
- Leave scaffold in cell culture medium until cell seeding.

3 Seed

These seeding densities are general guidelines only.

- 96-well plate: Add 10,000 cells suspended in 100-200 µL cell culture medium.
- 6- & 12-well plate: Add 50,000-100,000 cells suspended in 1 mL cell culture medium.

4 Exchange medium

- For long-term experiments semi-exchange the cell culture medium twice a week.

If you have received the scaffold as loose discs instead of pre-plated, make sure to remove them from their backing paper!

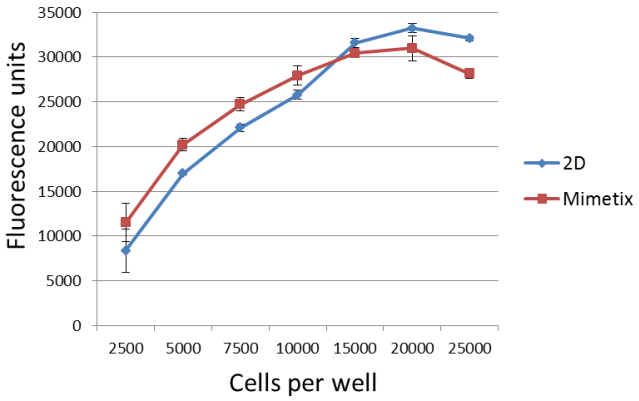
Assays used to assess cell viability

The Mimetix scaffold has been successfully used with a variety of cell viability reagents (for a complete list please refer to our website). Below we show 2 examples with CellTiter Blue and CellTiter Glo-based assays (Promega).

Both assays are perfectly suited for the Mimetix plate, as they can be performed directly in the plate without the need to transfer the cells.

CellTiter-Blue viability assay of HepG2 cells

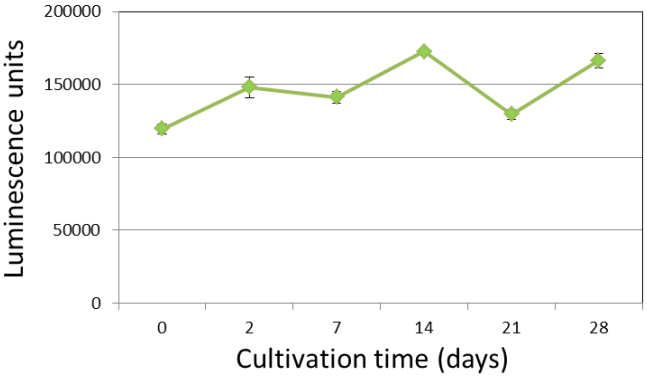
HepG2 cells were seeded at a range of densities in both Mimetix plates and standard 2D 96-well plates and incubated overnight prior to analysis. 10 µl of CellTiter Blue were added per well. Fluorescence was acquired on a plate reader at 580 nm after 90 minutes incubation at 37˚C.



A similar linear increase is observed with both plates up to 15,000 cells per well (n=6)

CellTiter Glo assay of HepG2 cells over 28 days

HepG2 cell viability was studied over a period of 28 days in a Mimetix 96-well plate. Cells were seeded at 10,000 cells/well, with ATP concentration quantified using CellTiter GLO (100 µl per well, Promega) after 90 minutes incubation at 37˚C in a plate reader following the manufacturer's instructions.



HepG2 in Mimetix are alive and metabolically active

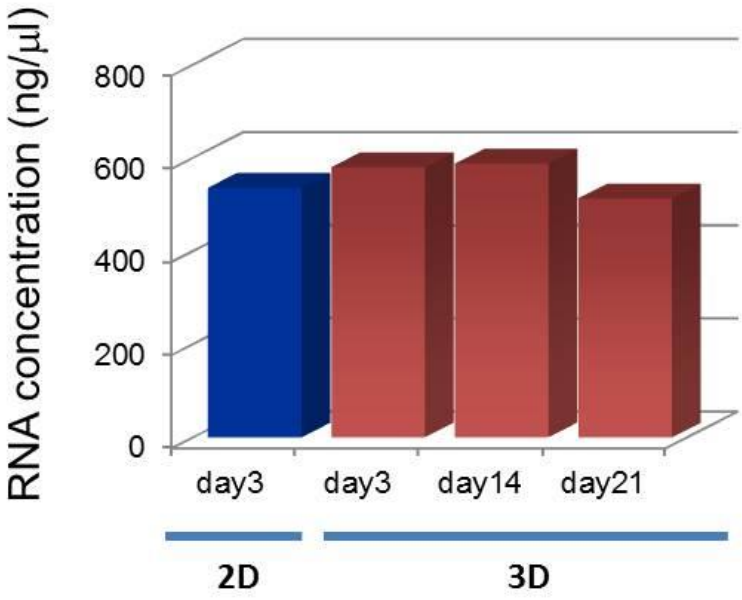
Optimised cell lysis for RNA preparation

Optimal cell lysis is obtained following these steps:

- Wash the cells twice with PBS
- Add 60 µl RLT (RNeasy kit, Qiagen) per well
- Leave the plate on ice for 5 minutes
- Shake the plate vigorously for 2 minutes in a plate shaker
- Leave the plate on ice for a further 30 minutes
- Carry on with the RNA isolation or freeze down the samples for later

Results for RNA preparation from HepG2 cells

Cells were seeded at 10,000 cells/well. RNA was prepared using the RNeasy kit (Qiagen) and quantified with the Nanodrop 8000 Spectrophotometer (Thermo Scientific).



The RNA recovery in 2D and Mimetix are identical.

Note: HepG2 cells do not proliferate in the Mimetix scaffold (see p.4), hence the RNA concentration is stable over time.

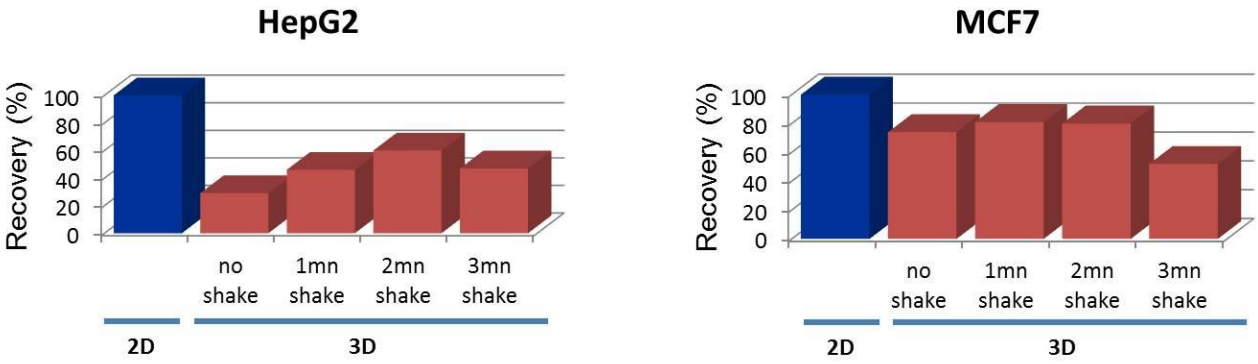
Optimised cell recovery

Optimal cell recovery is obtained following these steps:

- Wash the cells twice with PBS
- Add 40 µl 1xTrypsin per well
- Incubate the plate at 37˚C for 6 minutes
- Shake the plate vigorously for 2 minutes on a plate shaker
- Harvest the cells

Cell recovery data for HepG2 and MCF7 cells

Both cell types were seeded at 20,000 cells/well and grown over night before harvesting using different protocols. Results are given as a percentage of the cells recovered in a 2D plate.



Cells can be recovered from the Mimetix scaffold using trypsin. The recovery efficiency depends on the cell line. HepG2 are notoriously difficult to recover and 60% of the cells in Mimetix could be harvested compared to a 2D condition. Up to 80% of the MCF7 cells could be recovered.

The Mimetix scaffold is compatible with cell recovery using Trypsin.

Instructions for imaging cells in the Mimetix scaffold

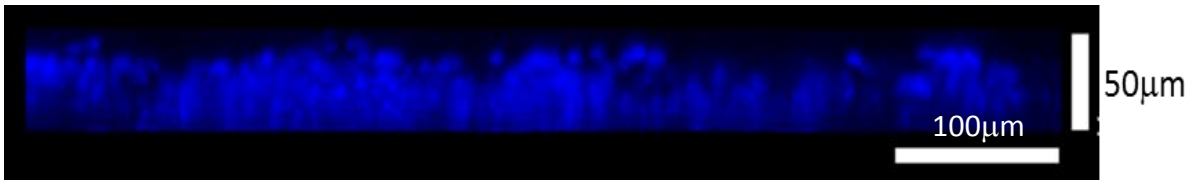
The Mimetix scaffold is compatible with imaging. The following protocol gives the best results:

- Wash the cells twice with PBS
- Fix the cells in 4% paraformaldehyde for 30 minutes
- Wash the cells twice with PBS
- Permeabilise cells with 0.1% Triton X-100 in PBS for 5 minutes
- Wash the cells twice with PBS
- Block using 0.5% BSA in PBS for 1h00
- Wash the cells twice with PBS
- Add primary antibody at the dilution recommended by the manufacturer and incubate for 1h
- Wash the cells twice with PBS
- Add the secondary antibody following the manufacturer's instructions
- Wash the cells twice with PBS

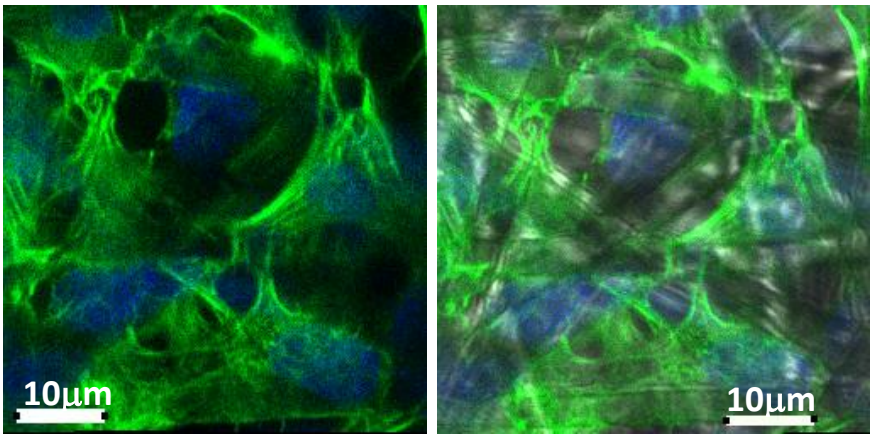
For DAPI and Actin staining, ReadyProbes® Reagents from Life Technologies (#R37606 and #R37110, respectively) work very well with the Mimetix scaffold.

Images in the Mimetix scaffold

HepG2 cells were grown for 21 days in the Mimetix scaffold, fixed in 4% paraformaldehyde and stained with DAPI (blue0 and Phalloidin (green). Confocal images were obtained using the Nikon Eclipse C1 microscope (objective x60) half way down the scaffold.

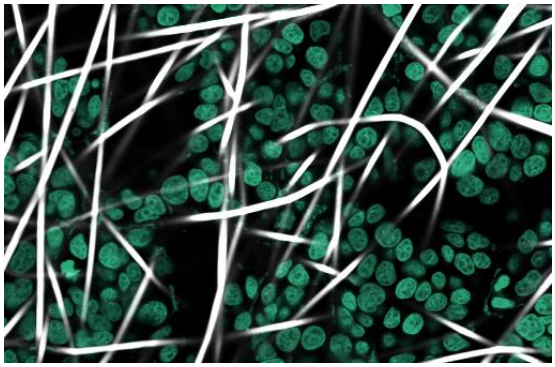


Cells populate the entire Mimetix scaffold

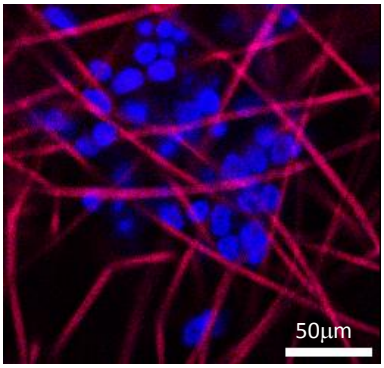


Cells form a 3D network and use the fibres to support themselves

We can incorporate rhodamine 6G into the fibres during electrospinning, as shown in the two examples below. Please contact us at info@electrospinning.co.uk for further details.



MCF7



HepG2

MCF7 breast cancer cells (stained with TO-PRO-3, green) are imaged 4 days after seeding.

HepG2 liver cancer cells (stained with DAPI, blue) are imaged 14 days after seeding.

Fibres are visible in white (left) or red (right).

Workflow comparison: 3D Mimetix scaffold vs. 2D

Workflow	2D	Mimetix® scaffold
Preconditioning	Not necessary	Wet with 20% ethanol, 2 subsequent washing steps with PBS and leave in culture medium.
Coating	Possible	As easy as 2D. Larger amounts of the coating agent might be required.
Cell seeding		Depends on cell type. Using a similar cell number for seeding than in 2D is a good starting point, but ideally a range of different seeding densities should be tested.
Medium exchange	Easy	As easy as 2D
Plate reader analysis	Easy	Fluorescence and Luminescence-based assays: as easy as 2D Absorbance-based assays: supernatant has to be transferred to a clear plate prior to read-out
Flow cytometry analysis	Easy	Same as 2D. Potentially longer incubation times/more rigorous shaking with lysis agent needed; please refer to protocol “Optimised cell recovery” for more information”.
Microscopic analysis	Easy	Confocal fluorescent microscopy: as easy as 2D Confocal brightfield microscopy: as easy as 2D Standard optical microscopy: does not work well because of scaffold opacity
Ease of Automation	Easy	As easy as 2D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