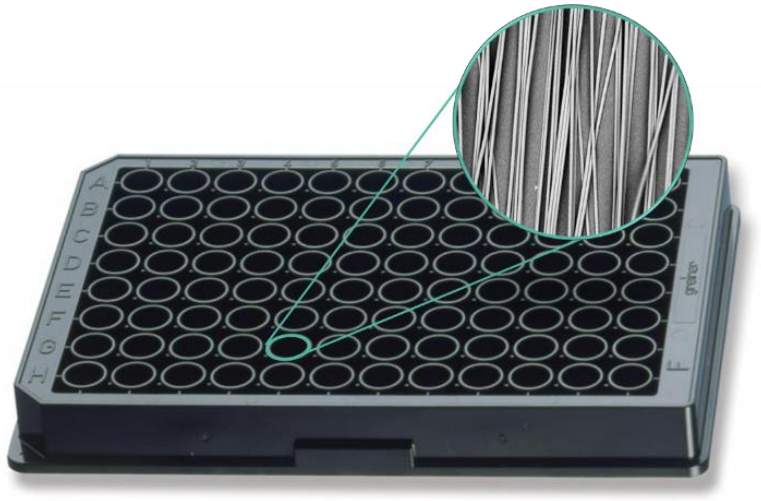
ELECTROSPINNING® COMPANY

Mimetix® Aligned Product Manual



MIMETIX
CELLS IN 3D

INSTRUCTION FOR USE

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1 Description

Mimetix® Aligned well plates contain highly aligned fibres and are ideal for the culture of cells which are influenced by topographical features. Aligned microfibres provide a physical structure for the 3D culture of cell from tissues such as the central nervous system, skeletal muscle and heart, where cellular orientation has been shown to play a significant role in the respective tissue functions and aims to induce these functions in vitro. They also provide an axon mimic for myelination by oligodendrocyte cells.

- Material: medical grade poly-L-lactide (PLLA)
- Fibre diameter: 2 µm
- Scaffold thickness: 2 to 4 µm
- Product use: For research use only. Not for use in diagnostic procedures.

2 Important Information

- Store at room temperature in the dark.
- Product shelf life: 2 years
- Compatible with industry standard automated handling and imaging equipment.
- Perform all procedures aseptically, in a laminar flow hood.
- Optimizing culture inoculation and incubation conditions may improve performance.

3 Mimetix Aligned Product Range

These plate formats are available as standard.

Products are supplied gamma irradiated (though can be supplied without).

Code	Plate format
TECL005	96 well plate
TECL006	12 well plate containing cell crown inserts
MBSS001	TECL006 but NOT gamma irradiated.

3.1 Plate Specifications

3.1.1 TECL005 - 96 well plate

The Mimetix aligned scaffold is welded into a Brooks Visionplate™. The frame is black, and the base is 190 µm polystyrene with excellent optical properties and high light transmission.

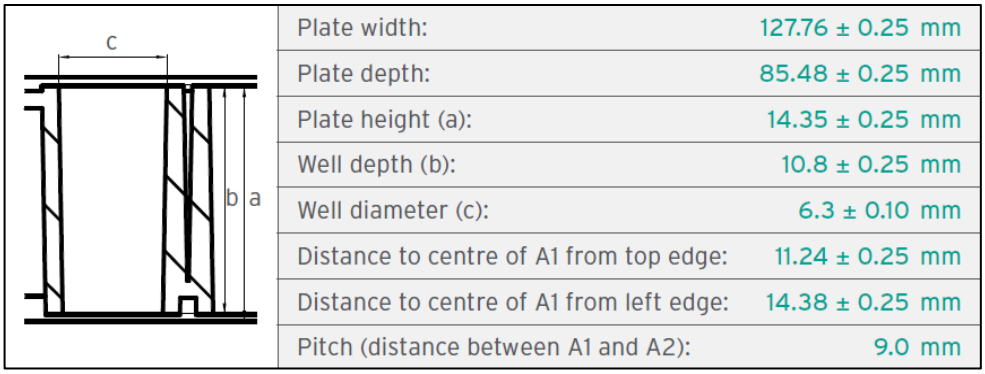

Plate width:	127.76 ± 0.25 mm
Plate depth:	85.48 ± 0.25 mm
Plate height (a):	14.35 ± 0.25 mm
Well depth (b):	10.8 ± 0.25 mm
Well diameter (c):	6.3 ± 0.10 mm
Distance to centre of A1 from top edge:	11.24 ± 0.25 mm
Distance to centre of A1 from left edge:	14.38 ± 0.25 mm
Pitch (distance between A1 and A2):	9.0 mm

3.1.2 TECL006/MBSS001 - 12 well plate containing cell crown inserts.

Cell crown dimensions:

Outer diameter of the body	17 mm
Inner diameter of the body	14 mm
Inner surface area	1.54 cm^2
Height of the body	12 mm
Inner diameter of the ring	17.5 mm
Height of the ring	3 mm

4 Protocol

The following protocol is written for use with TECL-006 but can be adapted for other product codes.

4.1 Preconditioning the Well Plates (Recommended)

Before seeding cells in Mimetix® well plates, soak the scaffolds in ethanol to increase the hydrophilicity.

Materials required (not provided)

- 70% ethanol
- Sterile water
- Poly-D-Lysin, 2mg/mL stock in sterile H_2O

Procedure

a. Add enough volume of 70% ethanol to cover the entire surface of the well in each plate.
b. Allow the ethanol to soak into the scaffold for 10 - 20 minutes.
c. Aspirate the ethanol from the sides of the well plate aseptically without touching the scaffold.
d. Immediately wash the scaffolds with sterile water (x3) (Do not let the fibres begin to dry).
e. Add enough poly-D-Lysin to cover the entire surface of the scaffolds and incubate for a minimum of 1 h to overnight in a cell culture incubator.

Note:

Scaffolds in non-sterilised plates (MBSS001) may be disinfected with 70% ethanol for 3 hours. In this instance, proceed with steps c – e of the preconditioning protocol. Please be aware that we cannot guarantee sterility of the scaffolds if this method is applied.

Thorough washing of the scaffolds after ethanol treatment is crucial to remove ethanol residues. Any remaining alcohol may affect the viability of cells.

4.2 Recommended Cell Seeding Protocol

The following protocol describes a generic culture protocol for cells. Examples for the culture of rat oligodendrocyte precursor cells are given in brackets.

Materials required (not provided)

- Sterile water
- Cells
- Cell culture medium
- 21-23 gauge needle
- Cell incubator

Procedure

a. Aspirate poly-D-Lysin and wash the fibres two times with sterile water to remove residual lysin.
b. Rinse once more with culture medium to remove the residual water adhering to scaffolds.
c. Before the addition of cells, fill each well with appropriate amount of pre-warmed (37º C) cell culture medium relevant for the cells being used.
d. Prepare cell suspension in a concentration appropriate to the cells used (e.g. 150,000 cells/mL)
e. Disperse any cell aggregates into single cells (e.g. using a 21- or 23-gauge needle)
f. Dispense cell suspension drop-wise to the inside of the cell crown insert in a concentration appropriate for the cells used (e.g. 30,000 cells per well).
g. Place cells into a cell culture incubator.
h. Perform medium changes regularly.

Note

Further information for the culture on oligodendrocyte precursor cells can be found here:

Marie E. Bechler. “A neuron-free microfiber assay to assess myelin sheath formation” (Chapter 6 in DOI:10.1007/978-1-4939-9072-6_5) 2019

4.3 Imaging

4.3.1 Microscopy – Fixing and Staining

Materials required (not provided)

- Blunt forceps with grip, for handling scaffolds
- PBS
- 3.7% formaldehyde
- 0.1% Triton X-100
- Primary and secondary antibody
- Fridge (if required)
- Nuclei stain (if required)

Procedure

a. Gently aspirate medium and wash once with PBS.
b. Aspirate and replace PBS with 3.7% formaldehyde/PBS for 15 minutes.
c. Remove formaldehyde and wash 3 times with PBS for 5 minutes.
d. Permeabilize cells with 0.1% Triton X-100/ PBS for 10 minutes.

e. Carefully push down each fibre scaffold to be sure that the scaffolds are sitting as low into the well as possible, gripping scaffolds only at the top to avoid disturbing the fibres.
f. Make primary antibody solution following the supplier's instructions. 0.75 mL of antibody solution per scaffold is sufficient to completely cover the fibres as long as each scaffold is sitting flat on the bottom of the 12 -well plate.
g. Fill several empty wells, if present, with PBS or water and seal the 12-well plate with parafilm to prevent evaporation.
h. Incubate primary antibody (e.g. at 4º C overnight).
i. Wash three times with PBS for 5 minutes to remove unbound antibody.
j. Prepare secondary antibody solution following the supplier's instructions. Add 0.75 mL of the solution to each scaffold.
k. Incubate scaffolds with secondary antibody (e.g. 1 h at room temperature) and wash the unbound antibodies with PBS twice.
l. Apply nuclei stain if necessary.

4.3.2 Mounting the Fibre Scaffolds onto Glass Microscope Slides

Materials required (not provided)

- Forceps
- Microscopy glass slides
- Paper towels or Kimwipes
- Dark storage box for glass slides
- Mounting medium
- 13 mm glass coverslips
- Pipette tip (preferably 1000 µL tip)
- Scalpel

Procedure

a. Set out a sheet of paper with the appropriate number of glass slides (2 slides per scaffold).
b. Have an appropriately sized box or cover to keep the scaffolds dark in between and after setting onto microscope slides.

Note: The following steps should be performed for each scaffold once at a time. Excessive residual PBS prevents proper hardening of the mounting medium. Avoid touching fibres or dislodging the ring on the bottom of the scaffold.

c. Add a drop (approx. 20 µL) of mounting medium to the slides for each scaffold.
d. Carefully remove the fibre scaffolds from the well and blot the rim of the scaffold onto a paper towel or a Kimwipe to remove excess PBS.
e. Place the fibres onto the drop of mounting medium. Working quickly, cover with two or three drops of additional mounting medium to completely cover the surface of the fibres. Immediately proceed to the next step.
f. Place a 13 mm coverslip inside the fibre scaffold and press down gently to sandwich the fibres between the coverslip and the glass slide.
g. While holding the top of the scaffold, use the back of forceps to push down the ring surrounding the outside of the scaffold. Gently press the coverslip down again with the pointed end of forceps to make sure that no air bubbles underneath and that the coverslip sits closely above the fibres. Carefully let go of the scaffolds and cover the slide to keep dark until it sets (Approx. 1-2 days to completely dry).

Note: Be extremely cautious not to bump the scaffolds at this stage and assure that the slide lies completely flat.

h. To remove the plastic scaffolding gently hold the coverslip in place with the back of a P1000 pipette tip while lifting the plastic scaffold upwards. The scaffold and the ring should both come off from the slide. If there is a lot of resistance, do not apply excessive force (as this risk cracking the coverslip), instead run a scalpel along the inside between the coverslip and the scaffold.
i. Once the scaffolding has been removed, a scalpel can be used to trim away excess hardened mounting medium from around the coverslip.
j. Store slides in the dark at 4º C short term or at -20º C for longer-term storage.