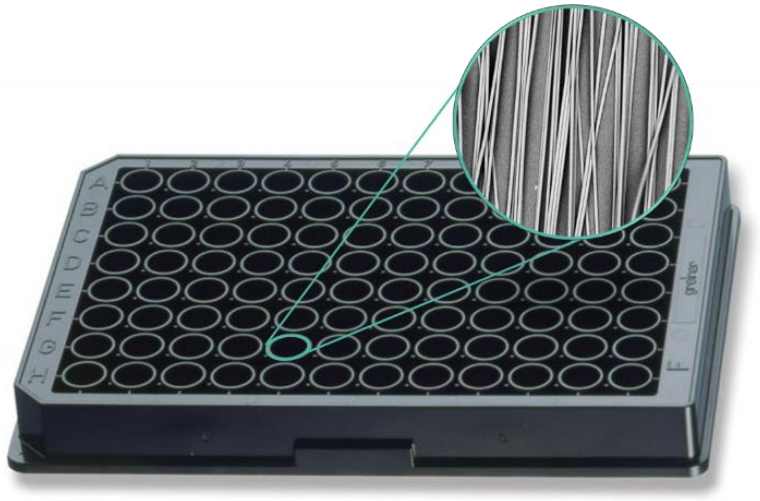
ELECTROSPINNING® COMPANY

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 in 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. In multiwell plates (96, 384) the aligned fibres are laser-welded into standard plate frames on a polystyrene base with excellent optical properties and high light transmission.

Code	Plate format
TECL005	96 well plate
TECL006	12 well plate containing cell crown inserts
TECL014	384 well plate
TECL015	384 well plate; fibres contain rhodamine

a. Plate Specifications

i. 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.

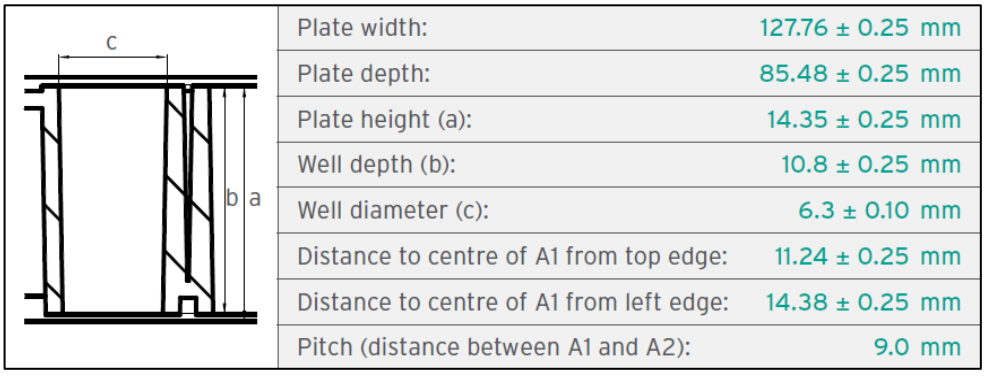

Plate width:	127.76 ± 0.25 mm
Plate depth:	85.48 ± 0.25 mm
Plate height (a):	14.35 ± 0.25 mm
Well depth (b):	10.8 ± 0.25 mm
Well diameter (c):	6.3 ± 0.10 mm
Distance to centre of A1 from top edge:	11.24 ± 0.25 mm
Distance to centre of A1 from left edge:	14.38 ± 0.25 mm
Pitch (distance between A1 and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

ii. TECL006 - 12 well plate containing cell crown inserts.
iii. TECL014 – 384 well plate

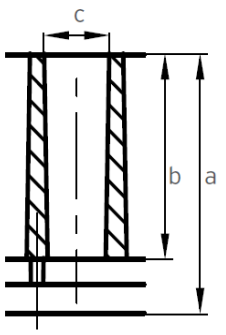

Plate width:	127.76 ± 0.25 mm
Plate depth:	85.48 ± 0.25 mm
Plate height (a):	14.35 ± 0.25 mm
Well depth (b):	11.35 ± 0.25 mm
Well diameter (c):	3.70 ± 0.10 mm
Distance to centre of A1 from top edge:	8.99 ± 0.25 mm
Distance to centre of A1 from left edge:	12.13 ± 0.25 mm
Pitch (distance between A1 and A2):	4.50 mm

4. Protocol

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

a. Materials Required (Not Provided)

- 70% ethanol
- Blunt forceps with grip, for handling scaffolds
- Sterile water
- Poly-D-Lysine (PDL), 2mg/mL stock in sterile H_2O
- 21 - gauge and 23 - gauge needles
- 2 ml syringes
- Sterile conical tube
- Cell culture incubator
- Complete cell culture media

b. Preconditioning the Well Plates (Recommended)

- Before seeding cells in Mimetix® well plates, soak the scaffolds in ethanol to increase the hydrophilicity.
- Add enough volume of 70% ethanol to cover the entire surface of the well in each plate.
- Allow the ethanol to soak into the scaffold for 10 - 20 minutes.
- Aspirate the ethanol from the sides of the well plate aseptically without touching the scaffold.
- Immediately wash the scaffolds with sterile water (x3) (Do not let the fibres begin to dry).
- Add enough PDL to cover the entire surface of the scaffolds and incubate for a minimum of 1 h to overnight in a cell culture incubator.

c. Recommended Cell Seeding Protocol

- Aspirate PDL and wash the fibres two times with sterile water to remove residual PDL.
- Rinse once more with myelin medium to remove the residual water adhering to scaffolds.
- Fill each well with appropriate amount of pre-warmed (37º C) myelin media in preparation for the addition of the cells.
- Collect oligodendrocyte precursor cells and triturate cells with 21 - gauge needle followed by 23 - gauge needle, if necessary, to break into single cells.

- Grow cells in appropriate media and culture conditions. Harvest cells and centrifuge at x300g for 5 minutes to obtain cell pellet. Resuspend cells up to 150,000 cells/mL in myelin medium. Proceed immediately to plating to prevent cells from settling and clumping.
- Add required quantity of rat or mouse OPCs dropwise to the inside of the cell crown insert, above the fibres.
- Place cells into a cell culture incubator at 37°C, 7.5% CO_2 and 95% humidity.
- Change media with fresh myelin medium every 2-3 days.

5. Imaging

a. Microscopy – Fixing and Staining

- Gently aspirate medium and wash once with PBS.
- Aspirate and replace PBS with 3.7% formaldehyde/PBS for 15 minutes.
- Remove formaldehyde and wash 3 times with PBS for 5 minutes.
- Permeabilize cells with 0.1% Triton X-100/ PBS for 10 minutes.
- Make primary antibody solution, 0.75 ml per scaffold, in PBS. This volume is sufficient to completely cover the fibres as long as each scaffold is sitting flat on the bottom of the 12 - well plate.
- 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.
- Fill several empty wells, if present, with PBS or water and seal the 12-well plate with parafilm to prevent evaporation.
- Place flat at 4º C to incubate overnight with primary antibody.
- Wash three times with PBS for 5 minutes to remove unbound antibody.
- Prepare secondary antibodies, 0.75 ml per scaffold, dilute in PBS.
- Incubate scaffolds with secondary antibody for 1 h at room temperature and wash the unbound antibodies with PBS twice.
- Stain the nuclei (if necessary) in PBS for 10 minutes and wash in PBS once for 5 minutes.

b. Mounting the Fibre Scaffolds onto Glass Microscope Slides

- Set out a sheet of paper with the appropriate number of glass slides (2 slides per scaffold).
- 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.

- Add a drop (approx. 20 µl) of mounting medium to the slides for each scaffold.
- Carefully remove the fibre scaffolds from the well and quickly blot the rim of the scaffold onto a paper towel or a Kimwipe to remove excess PBS.
- 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.
- Place a 13 mm coverslip inside the fibre scaffold and press down gently to sandwich the fibres between the coverslip and the glass slide.
- 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.

- 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.
- Once the scaffolding has been removed, a scalpel can be used to trim away excess hardened mounting medium from around the coverslip.
- Store slides in the dark at 4º C short term or at -20º C for longer-term storage.