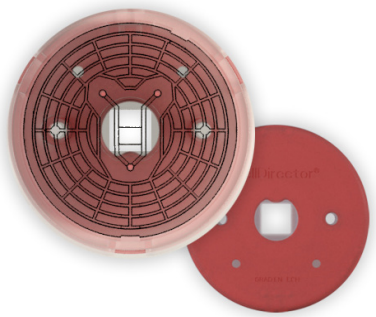
APPLICATION NOTE

gradientech

Dynamic antibiotic susceptibility testing



The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial substance that inhibits the visible growth of a micro-organism. The MIC value is usually obtained by an over-night incubation.

Using CellDirector® 3D, the MIC value for bacteria can now be determined in less than 3 hours. In addition, the existence of bacteria populations with different antibiotic susceptibility in mixed samples can be revealed, and quantitative information concerning bacterial responses in a continuum of sub-MIC concentrations is obtained.

Overview of experimental steps	Estimated time per step
1. Degas the blister package in a vacuum chamber	30 min
2. Dilute the bacteria sample to OD_{600} 0.1	5 min
3. Load bacteria-agarose mixture into CellDirector 3D	10 min + 10 min polymerisation
4. Start the experiment	10 min
5. Collect image data, preferably by time-lapse microscopy	≤ 3 h

CellDirector 3D overview

Precise fluid flows in CellDirector 3D generate **stable gradients in a 3D matrix**. The biological material - in this example bacteria - is injected together with an agarose matrix into CellDirector 3D. A linear concentration gradient of a single antimicrobial substance (or a combination of substances) is formed by diffusion through the polymerised gel. Many antibiotics/antimicrobial compounds are small and diffuse rapidly within the gel to form linear steady-state gradients within 2 hours.

Once the steady-state gradient has been established, the linear **gradient is maintained during the entire experiment**. Bacterial growth is directly visualised and analysed using microscopy and standard phase contrast imaging.

Loading of the bacteria-agarose mixture

The bacteria-agarose mixture that is injected into CellDirector 3D can contain air bubbles only visible through a microscope. To eliminate the risk of bubbles growing during 37 °C experiments, the blister package containing the CellDirector 3D assay can be placed in a vacuum chamber before sample loading.

The bacterial suspension is initially diluted to an OD600 of 0.1 and mixed with 1% low-melting agarose 1:1. Remove the blister package from the vacuum chamber and open it inside a laminar flow hood to take out the CellDirector 3D assay. Insert the pipette tip through the cross-shaped slit and inject 8 µl of the bacteria-agarose mixture into the cell culture chamber by reverse pipetting. Small air bubbles initially present within the agarose gel will rapidly disappear. Let the gel polymerise.

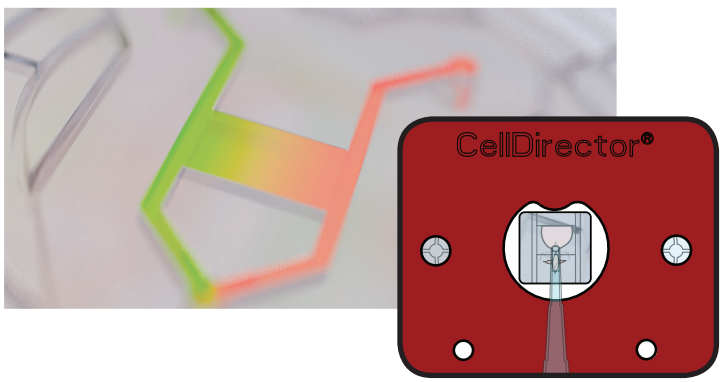


Figure 1. A steady-state antibiotic gradient is formed by diffusion through the polymerised bacteria-agarose mixture within CellDirector 3D.

Apply the antibiotic gradient

The fluid flows within CellDirector 3D are generated and controlled by an external syringe pump (Fusion 100, Gradientech) equipped with two syringes.

Syringe 1 is in this example filled with Mueller-Hinton medium only, whereas syringe 2 contains medium and an antibiotic. The tubes connected to the syringes are inserted into the CellDirector 3D assay, and the antibiotic gradient starts to build up by diffusion through the 4 mm wide agarose gel after starting the pump.

The susceptibility of *E. coli* K12 for ampicillin (0-20 µg/ml) was examined (Fig. 2), as well as the susceptibility of *Salmonella typhimurium* LT2 for streptomycin (0-20 µm/ml), see Fig. 3.

Data collection by time-lapse microscopy

CellDirector 3D was placed in an inverted microscope and images collected with a 2x phase contrast objective. Preferably, images can be collected using time-lapse microscopy to investigate the dynamic bacteria response over time. End-point imaging after the steady-state antibiotic gradient is established generates MIC values. Visualisation of bacteria subgroups with different degree of antibiotic resistance is possible.

Here, images were collected every 30 s for 4 h using a temperature controlled (37 °C) inverted microscope (TE2000, Nikon).

Image analysis and data interpretation

Bacterial growth or inhibition within the gel is quantified by measuring the grey scale intensity in the phase contrast images. Bacterial growth is detected by an increase in signal intensity. The intesity plot profile across the full gel width can be generated using ImageJ.

MIC values and the presence of intermediate or resistant subgroups are obtained from the intensity plots.

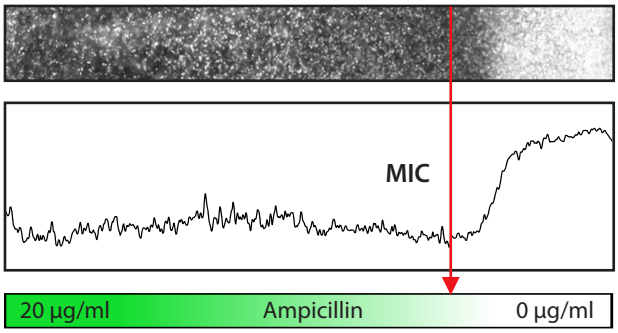


Figure 2. Phase contrast image of the 4 mm wide *E. coli*-agarose matrix within CellDirector 3D after 240 min. A linear steady-state gradient of ampicillin has formed and the response of the bacteria is shown by plotting the intensity values. High intensity values indicate living and dividing cells. CellDirector 3D generates a MIC value of 6 µg/ml (to be compared to 4-8 µg/ml from E-strip) [Ref 1].

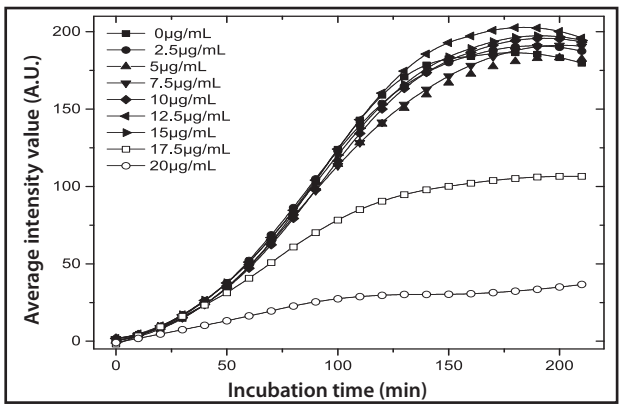


Figure 3. Growth curves of *Salmonella typhimurium* LT2 in CellDirector 3D in a gradient of streptomycin (0-20 µg/ml). Each concentration curve represents a position along the matrix. The intensity values reflects the numbers of living cells. CellDirector 3D generates a MIC value of 16 µg/ml (to be compared to 8-16 µg/ml from E-strip) [Ref 1].

Product information

PRODUCT	SUITABLE CELLS	APPLICATIONS	CATALOGUE #	SIZE
CellDirector® 3D	Adherent and non-adherent cells	Antibiotic susceptibility testing, chemotaxis or morphogenesis experiments of single cells or more complex tissues in 3D matrix	REF 10-001-10	10 assays/box

This Gradientech product is for research use only.

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REFERENCES
[1] Hou, Z. et al., *A microfluidic approach for dynamic investigation of the antibiotic susceptibility of bateria*, 24th Micromechanics and Microsystems Europe Conference, Espoo, Finland (2013)