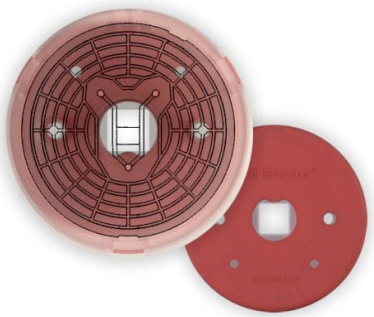
APPLICATION NOTE

GRADIENTECH

Induction of directional angiogenesis in mouse embryonic kidneys in a stable gradient of VEGFA



CellDirector® 3D enables high-quality analysis of for example angiogenic sprouting in complex tissues and organ culture models in response to very stable gradients of substances, for example growth factors. The dynamic responses of the 3D cell culture can be followed by time-lapse imaging for long periods of time. CellDirector 3D provides excellent cell culture conditions and maintains stable gradients throughout the entire experiment.

Overview experimental steps	Estimated time per step
Dissect embryonic kidneys from NMRI mice	According to the user's protocol
1. Degas the blister package in a vacuum chamber	30 min
2. Load cell-matrix mixture into CellDirector 3D	10 min + 60 min incubation
3. Starting the experiment	~ 10 min
4. Collect image data by time-lapse microscopy	Typically 12-48 h

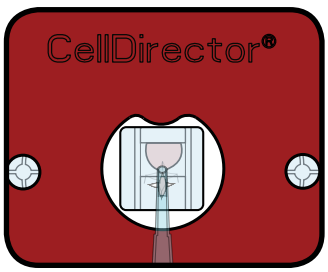
CellDirector 3D overview

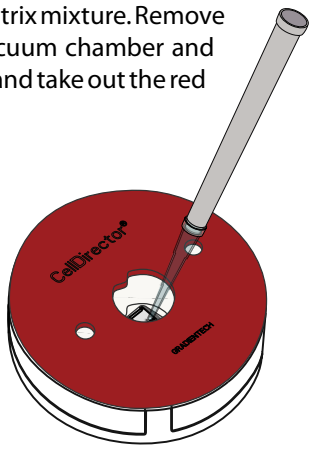
Precise fluid flows in CellDirector 3D generate **stable gradients in a 3D matrix**. The biological material - in this example an embryonic organ - is placed in an extracellular matrix, ECM , of choice inside CellDirector 3D. A linear concentration gradient is formed by diffusion through the ECM. The cells inside the ECM experience **no shear force**. Once the steady-state gradient has been established, the **gradient shape is maintained during the entire experiment**. The gradient shape is replicable between assays, provided the same gradient substance* and ECM is used in the experiments.

Degassing and loading cell-matrix mixture

The cell-matrix mixture that is injected into CellDirector 3D contains air bubbles only visible through a microscope. These small air bubbles will grow during longer experiments and affect the gradient formation, a phenomenon that is more apparent when the experiment is performed at 37 °C. To avoid this, the air bubbles in the matrix need to be removed prior to the start of the experiment as described below.

Place the blister package containing the CellDirector 3D assay in a vacuum chamber for 30 min before loading the cell-matrix mixture. Remove the blister package from the vacuum chamber and open it inside a sterile flow hood and take out the red CellDirector 3D assay.





Use a pipette and inject the embryonic kidney together with collagen I matrix by reverse pipetting. A total volume of 8 µl is injected by inserting the tip through the cross-shaped slit and injecting the matrix slowly.

* the gradient substance is defined as the molecule of choice used to build up the gradient

Any small bubbles within the matrix will rapidly disappear. Place CellDirector 3D in an incubator for 1 h to let the collagen polymerise.

Start the experiment and apply gradient

All **cell media** used for CellDirector 3D experiments are **placed in an incubator for degassing overnight** before the start of the experiment.
Two disposable syringes are used: syringe 1 is filled with cell media only, and syringe 2 with cell media containing the gradient substance. The 1 ml syringes that come with each CellDirector 3D assay are sufficient for 32 h experiments. Larger syringes can be used for longer experiments.

DMEM-Glutamax supplemented with 5 % serum and 1 % penicillin was used as cell media. Syringe 1 was filled with cell media only, and syringe 2 with cell media containing 50 ng/ml VEGFA. The syringes were placed in the syringe pump (Fusion 100, Gradientech) and the small vacuum pump (Vacuum 104, Gradientech) was connected to CellDirector 3D to reduce the risk of bubble formation during the experiment. Once fluid was exiting both tubes, they were inserted into the assay. A steady-state gradient was acheived after approximately 12 h.

Data collection by time-lapse microscopy

Time-lapse imaging was performed by collection of bright-field images every 20 min over a period of 48 h. Images were collected with 5× objective from the position where the embryonic kidney is placed. The captured image sequence showed that a gradient of 0-50 ng/ml VEGFA clearly induced directional vascularisation of the mouse kidney.

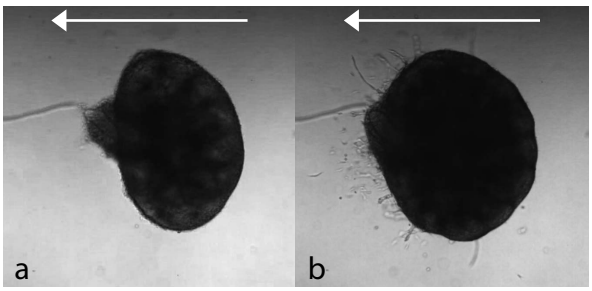


Figure 1. Embryonic mouse kidney placed in a gradient of VEGFA 0-50 ng/ml, a) at the start of the experiment, and b) after 48 h. The VEGFA gradient increases in the direction of the white arrow.

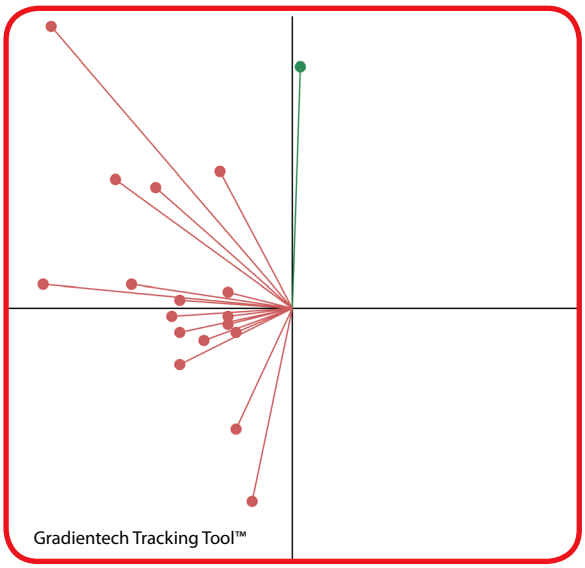


Figure 2. The plot shows the vascular sprouts that the kidney formed during the 48 h experiment. The sprout bases are positioned in the origo, and the end points indicate the position of the final sprout tips. Out of 18 visible sprouts, all but one pointed towards higher concentrations of VEGFA.
Sprouts were tracked with the Gradientech Tracking Tool™ software (freely available at www.gradientech.se).

Product information

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

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REFERENCES
Barkefors, I. et al. A fluidic device to study directional angiogenesis in complex tissue and organ culture models, Lab on a Chip, 9, 529-535 (2009)