zenCELL owl

Application Note - Cell intoxication assay

using the 24-channel microscope zenCELL owl

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Bacterial toxins play an important role for severe diseases such as pseudomembranous colitis. They are taken up into cells and enzymatically modify specific intracellular substrates which causes morphological changes in cells and results in clinical symptoms. Of particular interest are the molecular mechanisms of cellular uptake and modes of action of bacterial protein toxins like Clostridioides difficile toxins TcdA and TcdB.

Clostridioides difficile toxin TcdA is taken up into cells by receptor-mediated endocytosis and escapes from endosomes to the cytosol via a pH-dependent translocation step. In the cytosol, TcdA glucosylates Rho proteins, which act as molecular switches and regulate the actin cytoskeleton. Glucosylation inactivates Rho resulting in rounding up of adherent cells such as Vero cells (african green monkey kidney epithe-lial cell line). These morphological changes are specific and robust endpoints of intoxication and therefore used to monitor toxin activity (Figure 1).

For experiments, Vero cells were grown in 24-well plates at 37 °C. TcdA (5 ng/mL) was added and cell morphology continuously monitored at 37 °C using zenCELL owl. Images were taken every 30 min over a time course of 15 h (Figure 1). After 1 h, cells treated with TcdA show first effects of intoxication i.e. cell rounding. Increas-ing numbers of rounded cells were observed after 3 h and 8 h representing a typical intoxica-tion course of Vero cells with TcdA.

zenCELL owl incubator microscope is a very helpful tool to automatically document cellular morphological changes with a high temporal resolution. Operating zenCELL owl directly in the incubator provides undisturbed and continu-ous observation of the experiment at 37 °C. The software and handling of the hardware is very convenient and easy to use. zenCELL owl is a valuable tool to automate experimental pro-cesses, minimize the operator's time spent in the lab and maximize the number of conducted experiments.

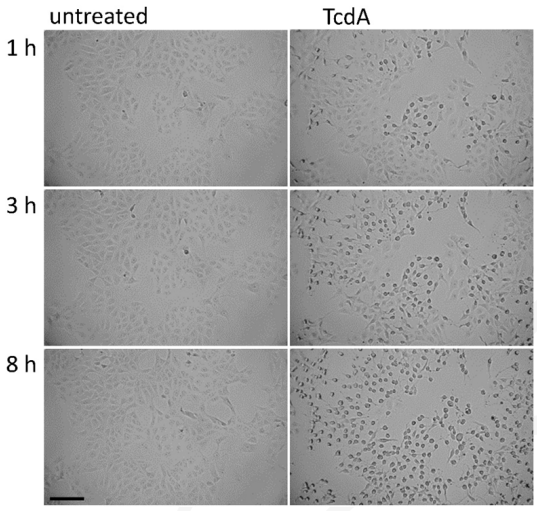

Figure 1: Cell rounding as a specific endpoint of intoxication with the bacterial toxin TcdA. Images are shown exemplarily after 1 h, 3 h and 8 h of intoxication with TcdA. Scale bar: 150 µm

zenCELL owl Live-Cell Imaging System

The zenCELL owl by innoME is a compact 24-channel microscope system for automated cell culture microscopy. The zenCELL owl fits easily into your standard incubator and monitors your cell culture continually. The device for your automated, objective and reproducible long-term monitoring.
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