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PERFORMING AN IN-VITRO CHEMOSENSITIVITY ASSAY ON THE CYRIS® ANALYSIS PLATFORM

ABSTRACT

The following exemplary protocol describes the preparation and run of an in-vitro chemosensitivity assay with chloroacetaldehyde (CAA) on adherent cells of the cell line MCF-7 on the CYRIS® analysis platform.

The CYRIS® platform is able to perform this automated assay with a deeper insight into the substance mode of action due to its ability to monitor the oxygen consumption rate (OCR), extracellular acidification rate (ECAR), cell impedance and microscopic images of the cells label-free in parallel and in real time. The cells are measured for 12 hours without treatment for baseline and for 24 hours under treatment with different CAA concentrations (0 µg/ml, 25 µM, 50 µM).

It is possible and may be necessary to adapt the protocol to other cell types, cell numbers, culture times and stock concentrations of CAA.

It is recommended to read the entire protocol once complete before the first test procedure to avoid problems with time management.

INTRODUCTION

An in-vitro chemosensitivity assay, as performed here, is a method used to analyze the cytotoxic effects of an antitumoral drug on living cancer cells in culture. These effects result from certain combinations of substance concentrations, treatment times, and cell types. In addition, conclusions can be drawn (depending on the assay performed) about the mechanism of action underlying the toxic effects.

The knowledge of such an antitumoral effect of a substance is the basis for a later clinical use and therefore can be important for drug development and candidate finding, too.

Cell-based in-vitro assays with a well-chosen cell model are fundamentally suited to replace or minimize animal tests, which are often required by law. In addition, they are able to reduce failure-related costs in drug development by early information about mode of action.

With this protocol the cytotoxic effects of CAA on the common human breast cancer cell-line MCF-7 can be tested. CAA is an alkylating agent which is metabolized in the liver of patients, treated with antitumoral-drugs such as cyclophosphamide or ifosfamide. Therefore, it is the cell culture-suitable form of chemotherapeutics commonly used for patients with breast cancer. To show dose- and time-dependent effects, in this assay a high effective (50 µM) and a medium effective (25 µM) concentration are tested against a control group (0 µM) over a treatment time of 24 hours.

ASSAY WORKFLOW

In preparation for the assay, the cells to be analyzed are seeded into the sterile sensor plate and cultured for 2–6 hours (depending on the cell type) under standard cell culture conditions. During this time, the CYRIS analysis platform is set up for the assay. For this purpose, the needed deep-well plate (DWP) storage vessels for baseline and treatment measuring are prepared with measurement media and the appropriate concentrations of substance to be tested, followed by acclimatization in the CYRIS® climate chamber. The pipetting robot is equipped with sterile tips. After the cells have adhered sufficiently, the culture medium on the cells is replaced by fresh measurement medium, the special fluidic lid is closed, and the sensor plate is inserted into the platform. By starting the corresponding sequence program, the chemosensitivity assay is performed automatically, and the acquired data is displayed in real time.

MATERIAL AND METHODS

CYRIS climate chamber

Set the environmental conditions in the CYRIS climate chamber to 37°C and 90% humidity.

Cell culture

MCF-7 breast cancer cells are maintained in DMEM high glucose supplemented with 5% FCS (culture medium). On the day of assay performance, the cells are detached and counted. A sample of 150 µl containing 50,000 cells (333,333 cells/ml) is seeded out into well 4-24 of the sterile and fully equipped sensor plate. Wells 1-3 are only filled with 150 µl culture medium (without cells), as they will be used as media control wells later. After seed out, the sensor plate should be closed by a standard multiwell-plate lid (not the special sensor-plate lid!) and left resting in the workbench for 20 minutes to allow the cells to sediment evenly. Afterward it is transferred to a standard cell incubator (37 °C, 5% CO_2, 95% humidity) for at least six hours to attach properly.

Reagents

Prepare a high concentrated stock solution of CAA in water. In this protocol we will use a 50 mM stock solution. If a different stock concentration is more useful for you, be sure to recalculate the needed volumes to create the final concentrations in measuring medium later.

Prewarm sterile measuring medium with 5% FCS to 37 °C in a water bath.

Preparation of DWP(1) for the 12-hour baseline measurement

Fill every well of one sterile DWP with 5 ml warm measuring medium and place directly into the CYRIS® incubator at position 5 (front left) for acclimatization. Cover it for transport from the sterile workbench to the incubator.

Preparation of DWP(2) for the 24-hour treatment measurement

Mix 56 ml warm measuring medium with 28 µl of the 50 mM CAA stock solution to create an CAA concentration of 25 µM. Fill wells 11-17 of a sterile DWP with 8 ml of this solution.

Mix 80 ml warm measuring medium with 80 µl of the 50 mM CAA stock solution to create an CAA concentration of 50 µM. Fill wells 1-3 (media control wells) and wells 18-24 of the same sterile DWP with 8 ml of this solution.

Fill wells 4-10 (cell control wells) of the same DWP with 8 ml of the pure measuring medium only and place the DWP directly into the CYRIS incubator at position 6 (middle left). Cover it for transport from the sterile workbench to the incubator.

Prepare waste DWP

Put an empty DWP into the CYRIS incubator at positions 2 (middle right) and 3 (front right) for wasted media.

Deep-well filling in list form

Measured function	Substance	DWP position	Volume measuring medium for mix	Amount of 50 mM CAA stock solution	Wells to be filled with mix	Volume of mix per well
Baseline	None	5	120 ml	/	1–24	5 ml
Treatment	CAA	6				
	00.0 µM		56.00 ml	/	4-10	8 ml
	25.0 µM		56.00 ml	28 µl	11-17	8 ml
	50.0 µM		80.00 ml	80 µl	1-3, 18-24	8 ml
Waste	None	2, 3	/	/	/	/

Fluidic

Equip the robot head with 24 sterile pipette tips and attach it to the robot arm in the CYRIS® climate chamber.

START AND EVALUATION OF EXPERIMENT

After six hours control the regular attachment of the MCF-7 cells In the sensor plate on an microscope. If cells are well attached, the culture medium on the cells in the sensor plate must be carefully removed (completely), by sucking It out over both well side chambers (micro channel connections). Be careful not to put the suction pipette in the middle well chamber with the cells attached, as this will result in loss of cells. Add 500 µl of warm measuring medium to each well, dividing the amount about equally between the two side chambers of each well. The medium should subsequently be evenly distributed in all 3 chambers by hydrostatic forces. Avoid air bubbles.

Lay down the sensor plate in a sterile workbench so that the contact pads point to the back (away from the operator). Take the sterile sensor plate lid and hold it with the right hand (disinfected gloves). To avoid air bubbles, which influence the measuring, raise the sensor plate with your left hand a few centimeters on the left side, so that it stands at an diagonally angle from top left to bottom right. Insert the lid by lowering it from the right side of the sensor plate to the left. First, place it loosely on the sensor plate. This allows the air bubbles to disappear. Make sure no air bubbles are trapped in the sensor chambers by examining the chamber from underneath. If no air bubbles are present, close the lid fully by gently pushing It down to the lowest possible position.

Now transfer the closed sensor plate Into the CYRIS platform. Two insert the test plate, the white ring at the robot base must be in the position “0”. Slide the sensor plate with the contact pins in the robot base and press it gently down into the blue corner holder. The plate should in this case be exactly aligned in parallel to the robot base and surrounded tightly at the frame corners. To lock the plate and close the impedance contacting, turn the ring counterclockwise to position “1”.

Now everything is set up for the performance of the experiment. Start ACE and choose an experiment protocol for full equipped plates with 12 hours premeasurement and 24 hours treatment (Like "12h-24h"). After starting the microscope initialization procedure and before the full start of the automatic modus, we recommend performing the "find focus" routine to get the best picture quality.

After this, press "start" to start the automatic mode. After start is selected, the platform executed the test procedure autonomously. During the whole assay, the OCR and the ECAR, as well as impedance and the morphology based on regular microscope images every 20 minutes, were recorded.

After the end of the test procedure, we recommend exporting the measured rates and impedance values and to perform a statistically analysis by grouping wells with the same concentration of CAA together. Through this a mean value and standard deviation of every group can be evaluated. In some cases, a normalization of the values to the time of the start of the treatment (12h) is advantageous to get a better overview.

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