1. Objective

This protocol describes the procedure for determining cell viability of cells cultured in the OrganoPlate® using a colorimetric WST-8 cell viability assay kit.

2. Background

The OrganoPlate® allows the culture of in-gel tissues (e.g. neuronal networks or liver cells), the culture of tubular tissues (e.g. endothelial or epithelial barriers), or combinations of both. The WST-8 cell viability assay makes use of a water-soluble tetrazolium salt, WST-8. The salt is reduced by cellular dehydrogenases to an orange formazan product that is soluble in culture medium. The amount of formazan produced is directly proportional to the number of living cells and can be detected using a plate reader.

3. Materials

- OrganoPlate® 2-lane or 3-lane (MIMETAS, 9605-400-B or 4003-400-B) with cultured cells
- Cell Counting Kit-8 (referred to in the protocol as “WST-8 cell viability kit”, Sigma, #96992)
- Cell culture medium and/or HBSS
- Multiwell plate reader (450 nm filter)

4. Background correction and controls

Before initiating the assay, the OrganoPlate® should be read on the plate reader so a background subtraction can be performed later. Moreover, in addition to the controls needed for your specific experimental setup, the following controls should be included to allow correct background correction:

1. No-cell controls in presence of medium and assay reagent (at least 2 chips) – for assessment of the background signal of a chip.
2. Medium-only controls containing cells but not assay reagent (at least 2 chips) – to check if the assay reagent affects the morphology of the cells; the readout is not required in the analysis.

In addition, the three types of controls below are necessary for **toxicology or compound** studies:

3. Negative/vehicle controls – chips that contain cells and medium with or without vehicle. These chips were not exposed to the experimental treatment (the compound of interest).
4. Treatment controls – chips that were exposed to the experimental treatment, but do not contain cells. Certain treatments or compounds may interact directly with the assay reagents resulting in aberrant readouts.
5. Positive controls – chips that contain a known compound or treatment at a concentration that will cause significant cell death.

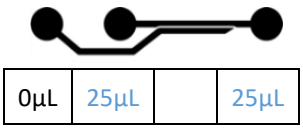
If the reagent is prepared in fresh medium, medium in control chips containing medium only should also be refreshed.

Note: In case the compound of interest modifies the enzymatic process underlying the conversion of WST-8 to formazan, a different viability assay should be employed. The "treatment controls" described in point 4 of the section above are used to assess if the compound of interest affects the WST-8 assay directly.

5. Procedure

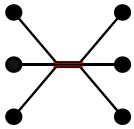
1. Dilute WST-8 from the kit (Sigma, #96992) 1:11 in cell culture medium or HBSS (cell type dependent) to obtain a WST-8 working solution
    a. Prepare 50 µL/chip and 100 µL/chip for the OrganoPlate® 2-lane and 3-lane, respectively
    b. Prepare 20% extra volume
2. Aspirate medium from all inlets and outlets of the OrganoPlate®
3. Add WST-8 working solution to all medium inlet and medium outlets of the chips according to the schemes below:

OrganoPlate® 2-lane



0µL	25µL		25µL

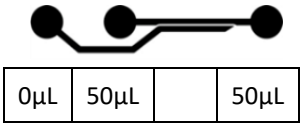
OrganoPlate® 3-lane



25µL		25µL
0µl		0µl
25µL		25µL

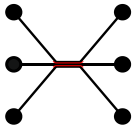
4. Incubate the OrganoPlate® at 37°C on the OrganoFlow®. The optimal incubation time depends on the type of cell and the type of culture and may require optimization.
    a. We recommend 20-30 minutes of incubation for tubular cultures, in which the cells are cultured against the ECM gel, and 2 hours of incubation time for in-gel cultures.
5. Next, remove the OrganoPlate® from the Perfusion Rocker™ and incubate the plate off the rocker for 5 minutes (at 37°C) to stop perfusion.
6. Measure absorbance at 450 nm using the multiwell plate reader.
7. Wash the OrganoPlate® chips for 5 minutes by adding HBSS (+ Ca/Mg) or medium (depending on cell type) using the volumes described in the images below. Place the OrganoPlate® on the OrganoFlow® during the wash.

OrganoPlate® 2-lane



0µL	50µL		50µL

OrganoPlate® 3-lane



100µL		50µL
20µL		20µL
50µL		50µL

8. After the wash, add medium to medium inlets and outlets to continue the culture or fix the plate

6. Data analysis

1. Calculate the average of the measurements for the medium inlets and outlets of each chip
2. Subtract the value of the **background control** (without cells) from all values
3. Plot desired graphs

MIMETAS product list

Cat. No.	Product Name
MI-AR-CC-01	OrganoReady® Caco-2
9605-400-B	OrganoPlate® 2-lane
4004-400-B	OrganoPlate® 3-lane 40
6405-400-B	OrganoPlate® 3-lane 64
6401-400-B	OrganoPlate® Graft
MI-OFPR-S	OrganoFlow® S
MI-OFPR-L	OrganoFlow® L
MI-OT-1	OrganoTEER®

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