Protocol for metabolic activity determination

WST-1

Cell viability, as a result of various enzymatic activities in live cultured cells, can be measured by using reagents / substrates that convert into measurable products (particularly optical density, for e.g. WST-1, Roche diagnostics).

* As per manufacturer's recommendation, add WST-1 reagent directly to the wells already seeded with hydroscaffold by adding 20µl reagent to 200µl medium in the well (dilution 1:10).

* Perform a blank with hydroscaffold without cells.

* Incubate at 37°C; 5% CO2.

* Perform optical density measurements at 440 nm by spectrophotometry after 30 minutes, 1h, 2h, 3h and 4h of incubation. This is based on the enzymatic activity of the cells tested.

* The sample should be returned to the incubator (37°C; 5% CO2) between each reading.

It is possible to use other commercially available kits for determining the metabolic activity such as:

MTT cell proliferation assay

* Renew culture medium and add MTT solution according to manufacturer's recommendations (final volume : 100µL per well). A blank should be done with hydroscaffold without cells.

* Incubate the plate at 37°C, 5% CO_2 for 3-4 hours.

* Add 100µL per well of the solubilization solution.

* Incubate the plate at 37°C, 5% CO_2. Time of incubation should be adapted according to manufacturer's recommendations and to your cell type.

* Read absorbance at 570nm.

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