ATPlite 3D

Luminescence Detection of ATP
From Cells Cultured in 3D

FOR RESEARCH USE ONLY

QUICK START GUIDE

A RESEARCH PRODUCT FOR RESEARCH PURPOSES ONLY

Instructions for use of product **6066943** (300 assay kit; 96-well format)

Kit Components

- 3 vials of Lyophilized Substrate Solution
- 1 x 20 mL of Substrate Buffer Solution
- 1 x 20 mL of Mammalian Cell Lysis Solution ('MCLS')
- 1 vial of Lyophilized ATP Standard Solution
- 1 x CellCarrier Spheroid ULA 96-well microplate (product # 6055330; 10 plates, 6055334; 40 plates)
- 1 x OptiPlate-96 HS (product # 6005330; 50 plates; 6005339; 200 plates)
- 4 x TopSeal-A (product # 6050185; 100 seals)
- 1 x Quick Start Guide

Storage

Buffer, MCLS and vials should be stored at 4°C. Microplates and TopSeal-A can be stored at Room Temperature.

Spheroid preparation

1. Spheroid cell cultures may be seeded and grown up directly in the CellCarrier Spheroid ULA 96-well microplate provided in the kit in the same way you would seed cells into a standard 96-well microplate.

2. For more details on seeding and growing spheroids see the "***User's Guide to CellCarrier Spheroid ULA Microplates"*** available on the PerkinElmer website.

3. A cell culture volume of 100 µL per well allows for the number of specified assay points to be obtained with this kit.

Reagent Preparation

1. Allow MCLS, Substrate Buffer Solution, and one vial of Lyophilized Substrate to reach room temperature (20 – 22°C). (One vial of Substrate is enough for one 96-well plate with 100 µL of culture volume per well.)

2. Reconstitute a vial of Lyophilized Substrate with 5 mL of Substrate Buffer Solution. Any remaining buffer solution can be stored at 4°C. Mix the contents of the vial gently by inversion and leave for 5 minutes.

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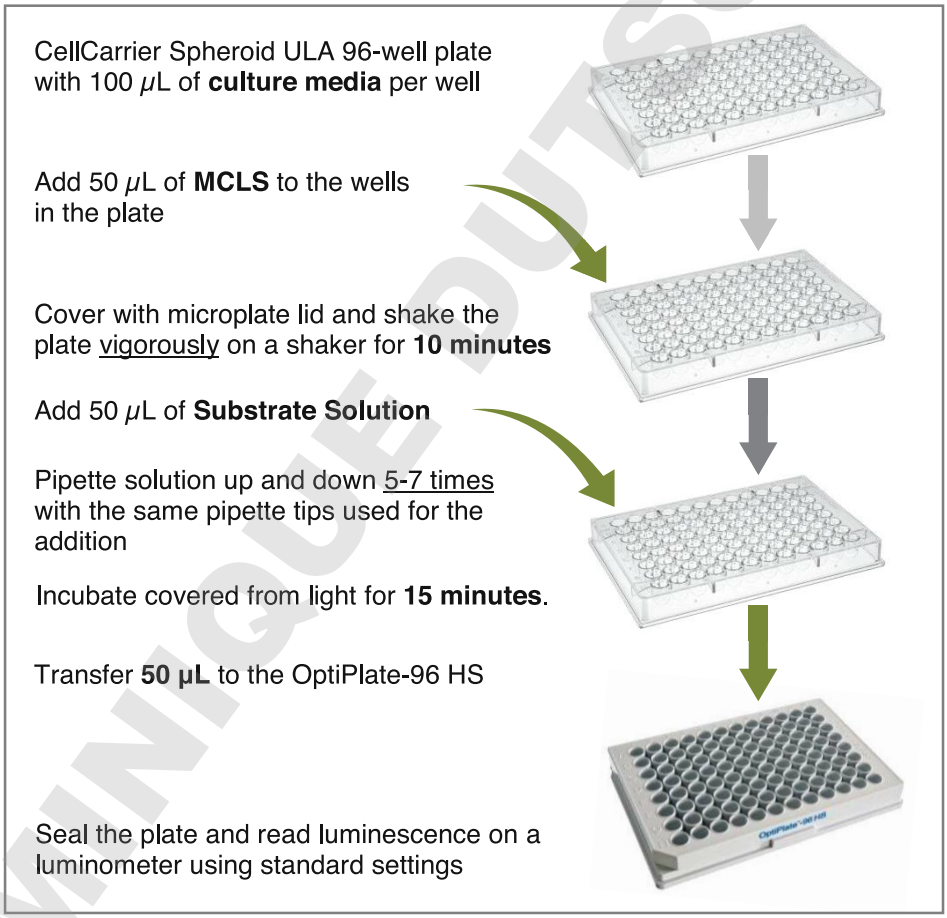
ATPlite 3D Protocol (for one 96-well plate)

1. Starting with 100 µL of culture volume (per well in the CellCarrier Spheroid ULA 96-well microplate), remove plate from incubator and add 50 µL of MCLS per well (preferably with an 8- or 12-multichannel pipette or automated liquid handler).
2. Cover the plate with a lid and move to an orbital shaker. Set shaker to a setting that will shake the plate as vigorously as possible without causing spill-over between wells in the plate. (On a "DELFIA Plateshake", which has a 1.5 mm orbital diameter, we find 700 RPM to be sufficient.)
3. Shake the plate for **10 minutes**.
4. Remove the plate from the shaker and add 50 µL of Substrate Solution per well preferably with an 8- or 12-channel pipette or automated liquid handler.
5. Mix vigorously (5 - 7 times) by pipetting 50 µL up and down with the tips angled towards the sides of each well. Larger and tighter spheroids benefit from more mixing to promote better penetration into the microtissue.
6. Incubate the plate at room temperature for **15 minutes** with the lid on the plate and covered to reduce exposure to ambient light.
7. Transfer 50 µL to an OptiPlate-96 HS.
8. Seal the plate with TopSeal-A and read luminescence under standard settings in a luminometer.

ATPlite 3D assay using 96-well plates



This product and/or its use is covered by the following patents and corresponding patent applications worldwide, owned by PerkinElmer Health Sciences B.V.: US Patent No. 6,503,723; EP Patent No. EP1117825B2.

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