QUICK START GUIDE

ATPlite 1step 3D

Single Addition Luminescence Detection of ATP
From Cells Cultured In 3D

A RESEARCH PRODUCT FOR RESEARCH PURPOSES ONLY

Instructions for use of product **6066736** (100 assay kit, 96-well format)

Kit Components and Storage

- 1 vial of Lyophilized Substrate Solution
- 1 x 10 mL of Substrate Buffer Solution
- 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 (product # 6005290; 50 plates; 6005299; 200 plates)
- 4 x TopSeal-A PLUS (product # 6050185; 100 seals)
- 1 x Quick Start Guide

Buffer and vials should be stored at 4°C. Microplates and TopSeal-A PLUS 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 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 Lyophilized Substrate Solution and Substrate Buffer Solution to reach room temperature (20 – 22°C). (One vial of Substrate Solution is enough for one 96-well plate with 100 µL of culture volume per well.)
2. Reconstitute the vial of Lyophilized Substrate Solution with 10 mL of Substrate Buffer Solution. Mix the contents of the vial gently by inversion and leave for 5 minutes.

ATPlite 1step 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 100 µL of the reconstituted Substrate Solution per well (preferably with an 8- or 12-multichannel pipette or automated liquid handler). 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.)
2. Shake the plate for 5 minutes.
3. Remove the plate from the shaker and incubate at room temperature for 20 minutes with the lid on the plate and covered to reduce exposure to ambient light. (You can shake the plate for the entire time if it works better for your workflow.)
4. 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.
5. Transfer 50 µL to the OptiPlate-96.
6. Seal the plate with TopSeal-A PLUS and read luminescence under standard settings in a luminometer.

ATPlite 1step 3D assay using 96-well plates

CellCarrier Spheroid ULA 96-well plate with 100 µL of **cell culture** per well

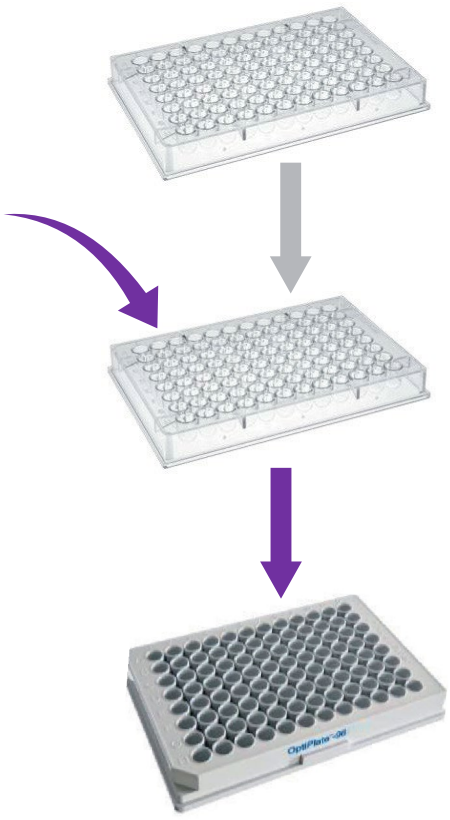
Add 100 µL of the **Substrate Solution** to the wells in the plate

Cover with microplate lid and shake the plate vigorously on a shaker for **5 minutes**

Remove from the shaker and incubate covered from light conditions for **20 minutes**

Pipette solution up and down 5 - 7 times and transfer **50 µL** to the OptiPlate-96

Seal the plate and read luminescence on a luminometer using standard settings



This product and/or its use is covered by the following patents and corresponding patent applications worldwide, owned by Revvity Health Sciences B.V.: US Patent No. 8,512,968; EP Patent No. EP2222870B1; China Patent No. CN101889095B; and Australia Patent No. AU2008319571B2.

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