

Measuring Hepatotoxicity in Primary Human Hepatocyte 3D Spheroids Using ViaLight® Plus Cytotoxicity BioAssay

Instructions for use

Safety Statements

These products are not for use in GMP manufacturing, nor human or animal in vivo use, including use as a diluent or as an excipient, or for diagnostic use.

These products are for research use *only*.

WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories, 5th edition](#). If you require further information, please contact your site safety officer or Scientific Support.

Culturing Primary Human Hepatocyte (PHH) 3D Spheroids

A [protocol](#) for long-term culturing of PHH 3D spheroids in 96-well plates can be found on the Lonza Bioscience website. This protocol also provides an example workflow for establishing a hepatotoxicity assay using acetaminophen to generate EC50 values.

This protocol is an optimization of the ViaLight® Plus Cytotoxicity BioAssay [Instructions for Use](#), specifically for use with PHH 3D spheroids in 96-well

plates due to the lower overall cell count per well in this model system compared to 2D PHH models or tumor-derived 3D spheroids.

Preparation of reagents

All work should be performed in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with 70% ethanol or isopropanol.

1. Hepatocyte Culture Medium (HCM Complete Medium)

- Transfer contents of HCM SingleQuots® Kit (Lonza part no. CC-4182) to HBM Basal Medium (Lonza part no. CC-3199) with a pipette and rinse each vial with medium. Store at 4°C for up to 1 month.

2. ViaLight® ATP Monitoring Reagent (AMR) Plus

- Add ViaLight® Assay Buffer into the vial containing the lyophilized ViaLight® AMR Plus until the vial is approximately 75% full. Screw the yellow cap back on and mix gently.
- Pour the reconstituted agent into the remaining assay buffer.
- Repeat steps a and b to ensure all lyophilized reagent has been transferred into the assay buffer.
- Allow the reagent to equilibrate for at least 30 minutes at room temperature to ensure complete rehydration.
- Excess prepared reagent can be stored at -20 °C and used within 3 months.

3. ATP Standards

- a. Prepare standards as shown in Table 1 using Lonza's 10 µM ATP standard.

ATP Standards		
Standard	Concentration (µM)	Formula
1	10 µM	300 µL of Lonza ATP standard
2	3.3333	100 µL of Standard 1 + 200 µL HCM Medium
3	1.1111	100 µL of Standard 2 + 200 µL HCM Medium
4	0.3704	100 µL of Standard 3 + 200 µL HCM Medium
5	0.1235	100 µL of Standard 4 + 200 µL HCM Medium
6	0.0412	100 µL of Standard 5 + 200 µL HCM Medium
7	0.0137	100 µL of Standard 6 + 200 µL HCM Medium
BLANK	0	300 µL HCM Medium

Table 1: ATP Standard Preparation

ViaLight® Plus Cytotoxicity BioAssay

NOTE: All work is to be performed in a laminar flow hood.

1. On the day of cell harvest and cytotoxicity assessment, begin by preparing the ViaLight® AMR Plus reagent, allowing it to equilibrate for at least 30 min at room temperature as described above.
2. Prepare the ATP standards as described above.
3. Remove 50 µl of medium from each well in the 96-well plate containing spheroids, pipetting at a 45° angle at the side of each well so as not to pipette out the spheroids.
4. Load 50 µL of each ATP standard in a separate white **assay plate**.
5. Add 25 µL of ViaLight® Plus Cell Lysis Reagent to each well (both samples in the original plate and standards in the assay plate).
6. Mix by gently shaking each plate.
7. Incubate plates at room temperature for 30 minutes.
8. Add 75 µL of ViaLight® AMR Plus to each well in both the samples in the original plate and standards in the assay plate.
9. Mix well by pipetting and transfer all volume (approximately 150 µL) from each well in the original sample plate to the 96-well white assay plate containing the standards. Ensure the standards have been mixed by pipetting as well.
10. Incubate for 2 minutes in the dark to allow full signal development.
11. Record luminescence on a luminometer.

- a. If the luminometer has a temperature control, it should be set to 22 °C, the optimal temperature for luciferase activity.

NOTE: Either a luminometer or a beta counter compatible with 96 well plates can be used for this assay.

- a. Luminometer read time: 1 second (integrated)
- b. Beta Counter:
 - a. Mode: out of coincidence or luminescence
 - b. Read Time: 1 second (integrated)

NOTE: Refer to the ViaLight® Plus Cytotoxicity BioAssay Kit [instructions](#) on the Lonza Biosciences website for further tips and troubleshooting.

Ordering Information

Catalog No.	Description	Size
HUCPG	Cryopreserved Primary Human Hepatocytes, Plateable (Verified for Spheroids)*	≥ 5 million cells
HUCPI	Cryopreserved Primary Human Hepatocytes, Plateable, DDI Qualified. (Verified for Spheroids)*	≥ 5 million cells
MCHT50	Human Hepatocyte Thawing Media	50 mL
CC-3199	HBM Basal Medium	500 mL
CC-4182	HCM SingleQuots® Supplements	1 kit
CC-3198	HCM Hepatocyte Culture Medium BulletKit®	1 kit
LT07-321	Lonza ViaLight® Plus BioAssay Kit	10,000 test kit
LT07-121		1,000 test kit
LT07-221		500 test kit
LT27-008	Lonza ATP Standard	5 mL

* **Ask for lots Verified for Spheroids:** Lonza routinely screens plateable hepatocyte lots for spheroid formation potential. To learn which lots are characterized for spheroid formation, contact Technical Support at scientific.support@lonza.com or scientific.support.eu@lonza.com.

96-well ultra-low attachment plates (Corning® CLS7007) mentioned are a product of Corning®.

96-well white luminescence plates (Corning® 3912) mentioned are a product of Corning®.

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