Lonza

Guide to Spheroid Formation Using Verified for Spheroids™ Human Hepatocytes

Use this protocol for spheroid formation with our Verified for Spheroids™ Human Hepatocytes to support your next steps towards more physiologically relevant liver cell culture systems.

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Introduction

The liver is the body's main site of metabolism of most small molecule drugs and consequently, *in vitro* models that accurately replicate liver function are highly desirable. It has recently been shown that primary hepatocytes, the main functional cells of the liver, can self-assemble into small spheroids when cells are placed into low-adherence plates or hanging liquid drops.[1] The formation of these spheroids improves the in-vivo-like response and increases the life span of the hepatocytes.

In this Technical Note, we describe the development of a simple spheroid formation protocol using our Verified for Spheroids™ Human Hepatocytes, to support researchers desire to implement hepatocyte spheroids as a routine model for *in vitro* metabolism, disease modeling, and toxicity testing.

Materials

1. Primary Verified for Spheroids™ Human Hepatocytes (Lonza, cat. no. HUCPI or HUCPG)
2. HCM™ BulletKit® (Lonza, cat. no. CC-3198)
3. Fetal Bovine Serum (FBS, Hyclone, cat. no. SH30071.03)
4. 1M HEPES Buffer (Lonza, cat. no. 12509079)
5. Human Cryopreserved Hepatocyte Thawing Medium (Lonza, cat. no. MCHT50)
6. 96-well Corning U-bottom ultra-low attachment (ULA, Sigma, cat. no. CLS7007)

Protocol

1. To formulate HCM™ Hepatocyte Culture Medium, transfer the contents of the HCM™ SingleQuots® Kit to HBM™ Basal Medium with a pipette, and rinse each vial with medium. Store at 4°C for up to 1 month.
2. Make complete Spheroid Formation Medium (SFM) by adding the following supplements to HCM™ Medium: FBS (final 20%) and HEPES Buffer (final 25mM). Store at 4°C for up to 1 month.

Note: We tested the effect of various levels of serum addition to the media on spheroid formation. Results indicate that spheroids require a minimum of 10% serum to form, but 20% serum produces the most optimal spheroids (Figure 1).

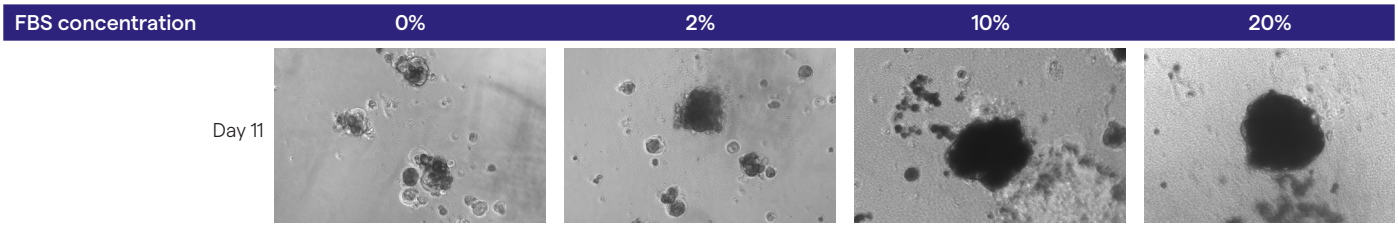

Figure 1.
At least 10% serum is required for spheroid formation. 3,000 primary human hepatocytes seeded in ULA U-bottom plates with serum at concentrations ranging from 0 – 20%. Optimal results are obtained when 20% serum is included in spheroid formation medium.

3. Thaw Verified for Spheroids™ Human Hepatocytes in thawing medium and centrifuge at speeds and times described in our Hepatocyte Thawing Protocol

Note: Each batch of Lonza's HUCPI and HUCPG are characterized for spheroid formation. Look for the Verified for Spheroids™ Specification in batch certificates or visit product web pages for up-to-date inventory.

4. Resuspend cells in 3 – 8 mL SFM and count cells using trypan blue and a hemocytometer.
5. Adjust cells to a final concentration of 1.0 x 10^4 – 3.0 x 10^4 cells/mL in SFM depending on the size of spheroid desired.

Note: We tested seeding between 1,000 – 5,000 cells per well. While all seeding densities produced spheroids, increasing the number of cells/well resulted in larger spheroids with cores beyond 500 µM. This enlarged size increases the risk for necrotic cores and therefore, we recommend using less than 3,000 cells/well when forming spheroids.

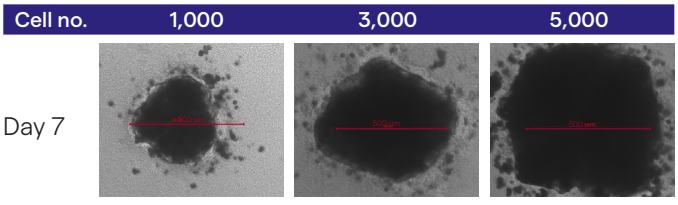

Figure 2.
Spheroids of different size. 1,000 to 5,000 primary human hepatocytes cultured in ULA U-bottom plates on day 7 after second media change. Spheroids reach size of 500 µM when 3,000 cells are used for spheroid formation.

6. To begin spheroid formation, add 100 µL cell suspension to wells of a U-bottom ULA 96-well plate and place in incubator at 37°C 5% CO_2. Incubate undisturbed for 120 – 168 hours.

Note: For extended cultures of spheroids, we recommend filling all outer edge wells with medium only to assist with evaporation issues. Any disturbance of the plates or mishandling during incubation can negatively affect spheroid formation.

7. Once spheroids have formed, remove 50% of medium and add fresh complete HCM™ Medium every 2 to 3 days. More frequent media changes can facilitate faster removal of remaining serum used during spheroid formation.

Our Verified for Spheroids™ Human Hepatocytes are plateable human hepatocytes that are already qualified as general purpose (cat. no. HUCPG) or Interaction Qualified (cat. no. HUCPI) and further tested for forming a tight spheroid by 7 days in culture. In our extended studies, it was observed that there is some donor-to-donor variability in both the formation rate and longevity of hepatocyte spheroids.

Additional studies can be found in a separate White Paper that show certain hepatocyte lots can last in spheroid culture up to 28 days with continued high-level Cytochrome P450 activity. These efforts support the potential use of our Verified for Spheroids™ Human Hepatocytes for extended toxicology and DMPK studies and reduce the need for researchers to test different donors for spheroid formation.

References:

1 Bell CC, Hendriks DF, Moro SM, Ellis E, Walsh J, Renblom A, Puigvert LF, Dankers AC, Jacobs F, Snoeys J, Sison-Young RL. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. Scientific reports. 2016 May 4;6; 25187.

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