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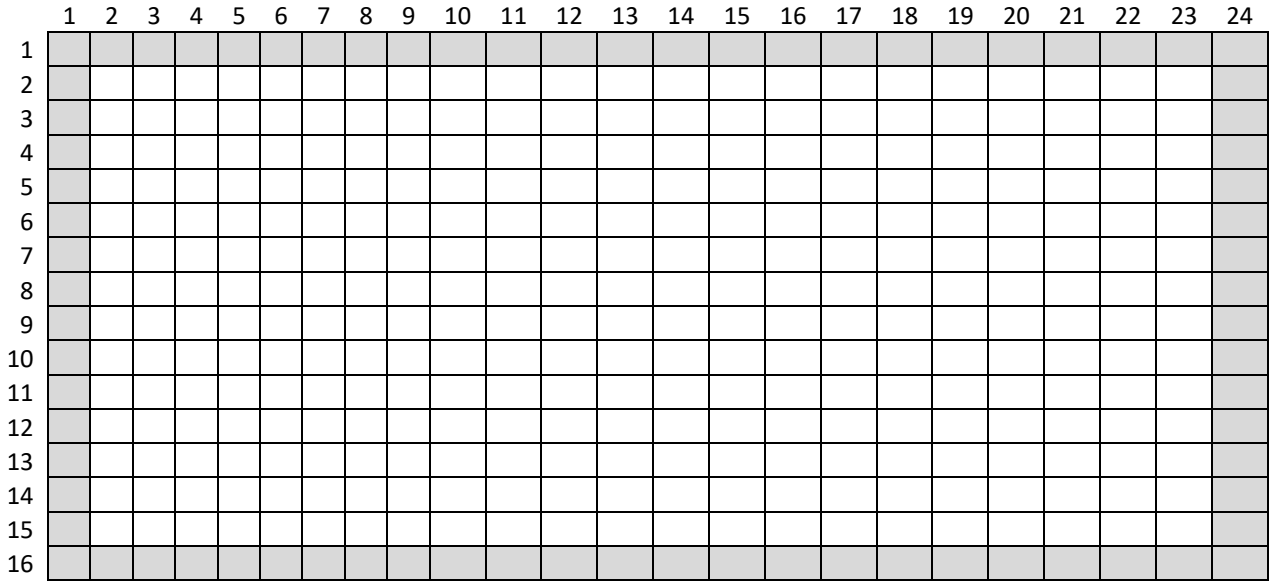
Spheroid 3D Culture Protocol for Human Hepatocytes

Hepatocyte Reagents and Materials	**Order Information**	**Cat #**
• Spheroid Qualified Human Hepatocytes	DLS SEE PCS	82006
• 384 well plate: Corning Spheroid microplate, black with clear bottom	Corning	3830
• 96 well plate: Corning Spheroid microplate, black with clear bottom	Corning	4520
• Universal Primary Cell Plating Medium-A, (UPCM-A™) 40 mL, with supplements	DLS	81070
• Universal Primary Cell Plating Medium (UPCM™) 50 mL	DLS	81016
• Hepatocyte Induction Medium, 50 mL (HIM™) (with Supplements)	DLS	81018

Plate map:

Fill outer wells with 50 µL PBS (grayed area in plate map) to create additional moisture to prevent the well from drying out



Laboratory Tools

Prior to initiation of the induction assay, ensure the Biological Safety Cabinet (BSC) is equipped with the following:

- Pipettes and appropriate sterile tips
- Disposable Pipetting Reservoirs, Sterile
- Ice bucket with ice
- 37°C incubator maintained at 95% balanced air and 5% CO_2

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Protocol

1. Use DLS Protocol - Thawing, Counting, and Plating Cryopreserved Plateable Hepatocytes to thaw spheroid qualified human hepatocyte lot

2. 96 well plates cell density: 40000 cells /mL, 4000 cells /100 µL (…/0.04)
   - Thaw UPCM-A in 37°C until a small sliver of ice remains, place immediately in ice
   - Keep UPCM on ice
   - Add 50 µL UPCM-A to 96 well plate to each well
   - Then add 50 µL 2000 cells/well with plating medium
   - Final cell density is 2000cells in 100 µL
   - Centrifuge plate at 200xg for 2 minutes
   - Be careful to move the plate to the incubator. This initial incubation should be for 7 days
   - After the initial incubation please change medium with HIM™ every two days
   - PLEASE DO NOT ASPIRATE TO CHANGE MEDIUM
   - Change medium by very gently and slowly taking out 50 µL of medium and adding back 50 µL medium
   - Avoid disturbing spheroid or touching the bottom of the well
   - Change medium until using or shipping.

3. For 384 well plates: cell density is 80000/mL, 4000 cells/50µl (.. /0.08)
   - Thaw UPCM-A in 37°C until a small sliver of ice remains, place immediately in ice
   - Keep UPCM on ice
   - Add 50 µL UPCM-A to 384 well plate to each well
   - Then add 25 µL 2000 cell/well with plating medium to 384well
   - Final 50ul, cell density is 2000 cell/50ul (this time just have 1000 cells /50ul/ well)
   - Centrifuge 200xg for 2 minutes
   - Be careful to move the plate to the incubator. This initial incubation should be for 5-7 days
   - After the initial incubation please change medium with HIM™ every two days
   - PLEASE DO NOT ASPIRATE TO CHANGE MEDIUM
   - Change medium by very gently and slowly taking out 25 µL of medium and adding back 25 µL medium
   - Avoid disturbing spheroid or touching the bottom of the well
   - Change medium until using or shipping.

We have almost more than 90% successful spheroids for one 384 well plate.

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