FAQ: Tips & Tricks for using Plateable Hepatocytes in In Vitro Assays

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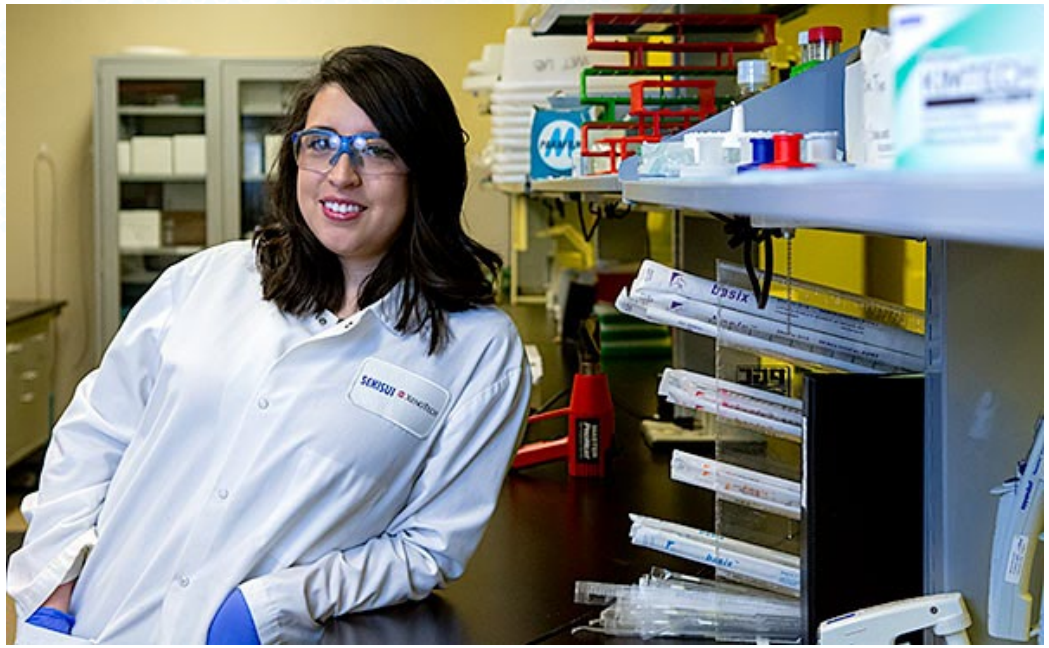


Overview: Frequently Asked Questions

- What are plateable hepatocytes used for?
- What do you consider to be an attaching lot?
- How do I choose which species to use?
- How do I know what concentration is best for seeding?
- What's the difference between CryostaX and traditional?
- How crucial is accurate cell count?
- How long can my cells sit before I plate them?
- Why do I have to wait until the next day to overlay rat hepatocytes?
- My 96 wells aren't working; what am I doing wrong?

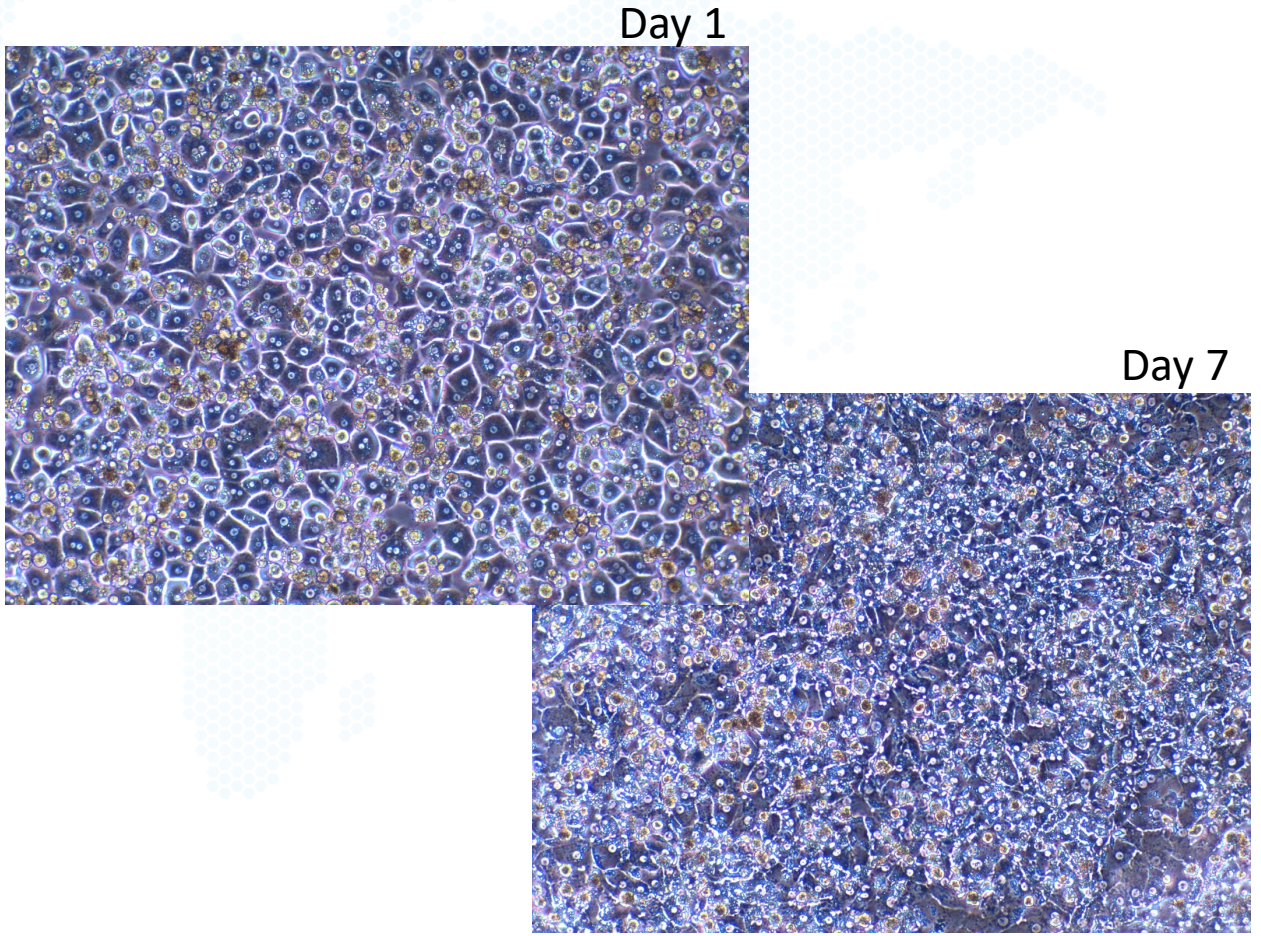
What are plateable hepatocytes used for?

- Induction
- Metabolism
- Cell biology
- Biochemistry
- Virology
- Parasitology

And so much more!

What do you consider to be an attaching lot?

- Attaching lots must form a monolayer that is AT LEAST 85% confluent.
- Cells must be able to survive throughout a 3-day induction period, but typically, cultures will last much longer.
- We monitor attaching lots through day 7.

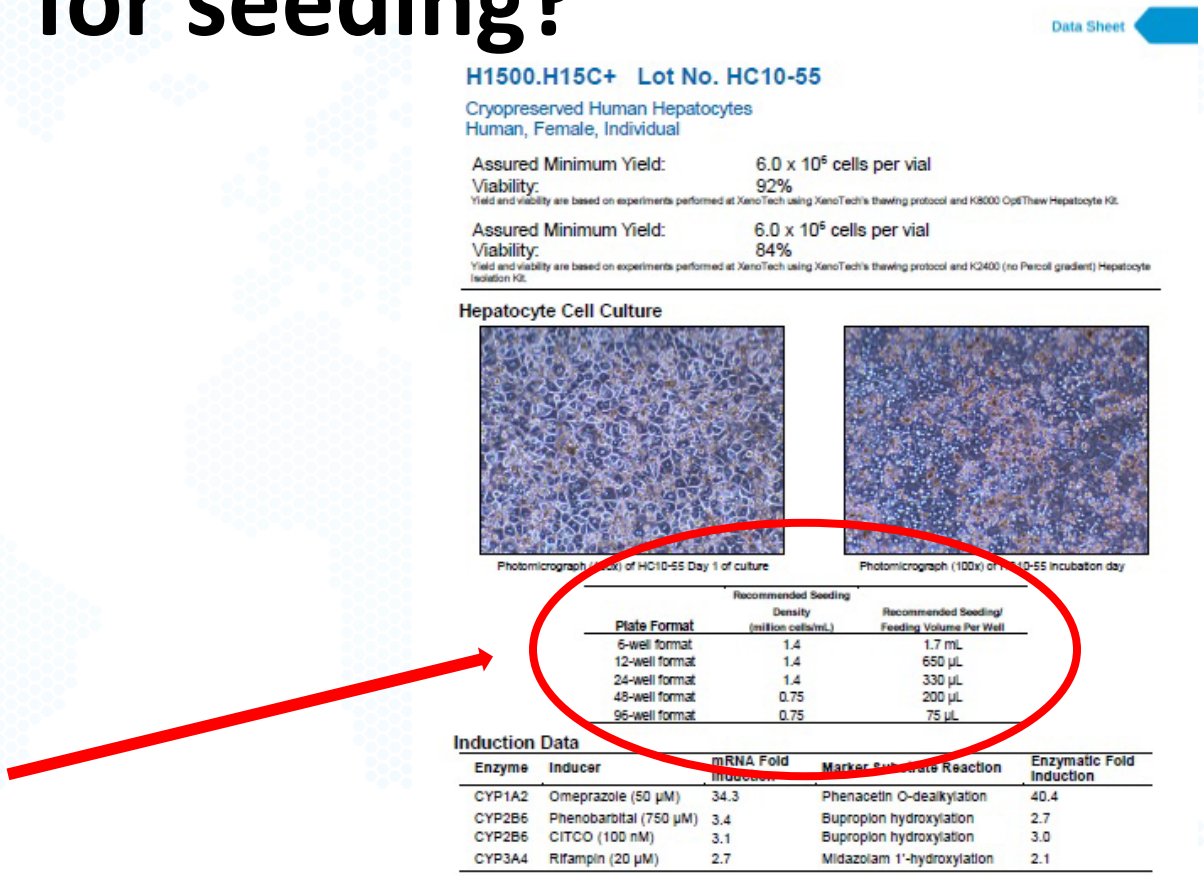


How do I choose which species to use?

- Animal hepatocytes from small, common laboratory animals are usually pretty consistent in activities.
- There is a huge difference in activity levels in human donors so choosing which one is best would depend on what is being looked at.
- WE CAN HELP!
  - Speak to our technical support specialists and we can help you choose.
    - +1.913.438.7450
    - info@xenotechllc.com

How do I know what concentration will be best for seeding?

- All hepatocyte lots are tested to optimize seeding concentration before ever being released for client use.
- All of this information is found on our product datasheet and is conveniently broken down by well format.



Data Sheet

H1500.H15C+ Lot No. HC10-55

Cryopreserved Human Hepatocytes
Human, Female, Individual

Assured Minimum Yield: 6.0 x 10^6 cells per vial
Viability: 92%
Yield and viability are based on experiments performed at XenoTech using XenoTech's thawing protocol and K8000 OptiThaw Hepatocyte Kit.

Assured Minimum Yield: 6.0 x 10^6 cells per vial
Viability: 84%
Yield and viability are based on experiments performed at XenoTech using XenoTech's thawing protocol and K2400 (no Percoll gradient) Hepatocyte Isolation Kit.

Hepatocyte Cell Culture

Photomicrograph (100x) of HC10-55 Day 1 of culture

Photomicrograph (100x) of HC10-55 incubation day

Plate Format	Recommended Seeding Density (million cells/mL)	Recommended Seeding/ Feeding Volume Per Well
6-well format	1.4	1.7 mL
12-well format	1.4	650 µL
24-well format	1.4	330 µL
48-well format	0.75	200 µL
96-well format	0.75	75 µL

Induction Data

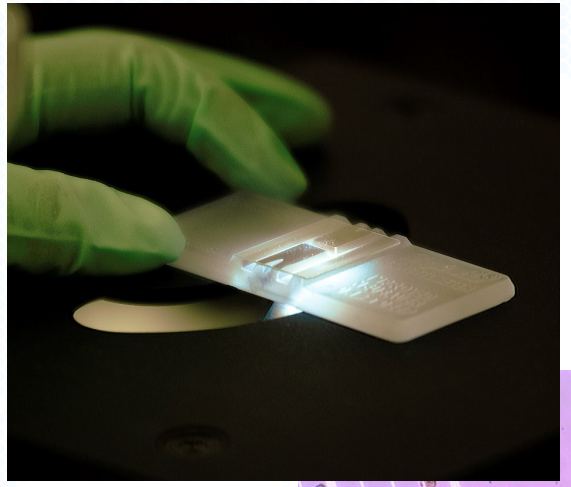
Enzyme	Inducer	mRNA Fold Induction	Marker Substrate Reaction	Enzymatic Fold Induction
CYP1A2	Omeprazole (50 µM)	34.3	Phenacetin O-dealkylation	40.4
CYP2B6	Phenobarbital (750 µM)	3.4	Bupropion hydroxylation	2.7
CYP2B6	CITCO (100 nM)	3.1	Bupropion hydroxylation	3.0
CYP3A4	Rifampin (20 µM)	2.7	Midazolam 1'-hydroxylation	2.1

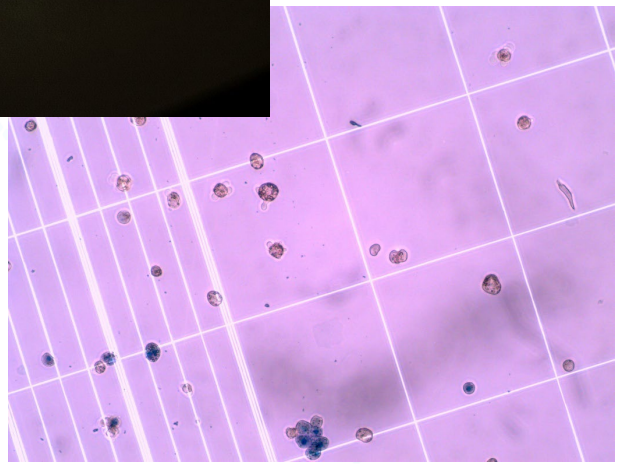
What is the difference between CryostaX and traditional vials?

- The only difference between CryostaX and traditional vials is the method of cryopreservation. This small change can make a HUGE difference in the outcome of your thaw.
- CryostaX vials are in pellet form and do not require a water bath when thawing. A common mistake when thawing is keeping hepatocytes in the water bath for too long. This can have detrimental effects on yield and viability. By eliminating the water bath, the chances of having a successful thaw greatly improve.

How crucial is an accurate cell count?

- Cell counts are essential because both under-seeding and over-seeding can lead to under-confluent cultures.
- Being consistent in your technique can ensure your counts are as accurate as possible.





How crucial is an accurate cell count?

- Hepatocytes settle quickly! The cell suspension should be resuspended throughout the process and always before removing a sample for counting.
- Take the cell count sample from the middle of the volume. This practice lowers the chances a pocket of cells is included in the count which would falsely elevate the yield.
- When adding the cell suspension to the counting tube, the pipette tip should barely break the surface of the counting solution so cells on the outside of the tip are not included in the count.
- Do not pipette up and down to mix! Instead, mix by rocking gently.

How long can my cells sit before I plate them?

- It is a good rule of thumb to get cells on a plate within 30 minutes of thawing.
- It is always helpful to have everything set up and have all tubes and plates labeled before ever removing the vials from cryostorage.
- Media should always be pre-warmed to 37 °C prior to starting any procedure. However, media and cells do not need to be kept in a warmer while working with them and room temperature conditions are fine.

Why do I have to wait until the next day to overlay rat hepatocytes?

- Rat hepatocytes have a natural gapping pattern that form and this can make cultures appear under-confluent.
- Waiting 18-24 hours post-seeding gives the cells more opportunity to flatten and form a confluent monolayer.
- Overlaying is important because sandwich cultures allows cells to regain polarity, form canalicular networks, and retain morphology throughout the culture period. Hepatocytes also retain more *in vivo*-like properties.

My 96 wells aren't working; what am I doing wrong?

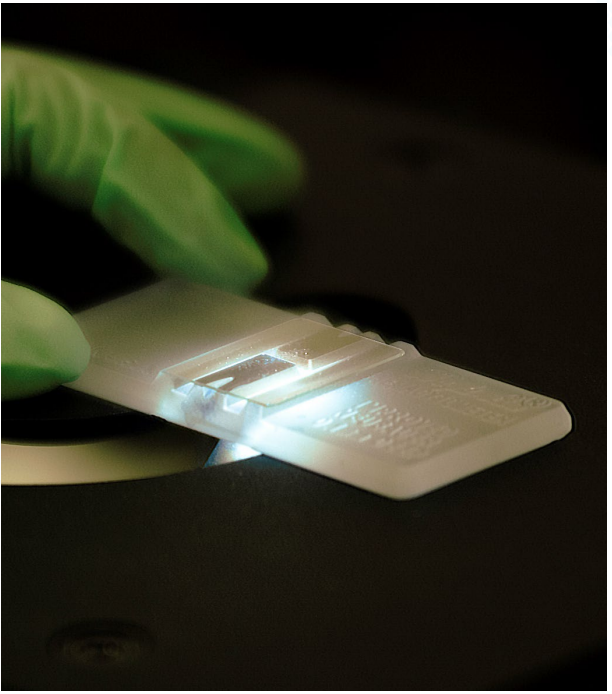
- Well-to-well variability is a common issue in 96 well plates because the well size is so small, small differences in seeding numbers can make a big difference in monolayer formation.
- We recommend reverse pipetting.
  - Go all the way down to the second stop before pulling up any cell suspension.
  - When dispensing into the wells, only go down to the first stop.
  - This is important because it eliminates the chance of air bubbles being introduced into the wells.
  - Air bubbles disrupt monolayer formation and can make entire wells unusable in the 96 well format.

My 96 wells aren't working; what am I doing wrong?

- Manual multichannel pipettes are preferred.
  - If electronic multichannels are used, the speed should be turned down and only half the plate should be dispensed at a time. Cells can settle in disposable tips and this can lead to uneven numbers of cells being seeded into wells.

Thank you for watching!

Please contact one of our Test Systems specialists to find out more or to place an order for hepatocytes.



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