Important Considerations

For Hepatocyte Cell Culture

This protocol is suitable for the thawing of suspension and plateable cryopreserved hepatocytes. Please read through this entire protocol before attempting this procedure. The health of the hepatocytes is dependent upon following the protocol carefully. *Please supplement the basal hepatocytes plating and maintenance media with the provided supplement before use. The thawing media needs no supplementation and can be used as is.*

Procedure for Thawing

1. Warm the Hepatocyte Thawing Media in a 37°C waterbath.
2. Once the thawing medium is warmed, disinfect it (70% ethanol wipe or spray) and transfer it to the biological safety cabinet (BSC) (or alternative designated area).
3. Quickly remove the cryopreserved hepatocytes from their storage location. Vertically submerge as much of the vial as possible, up to the cap, in the waterbath. It is important to make sure the cap of the vial stays above the waterline.
4. Thaw the vial for approximately 2 minutes. The vial will thaw from the outside to the inside; you may see a spindle form and shrink as the vial thaws.
5. Once thawed, disinfect the vial and transfer it to sterile biosafety cabinet (BSC). Pipette hepatocytes into a 50 mL conical tube of thawing medium. You may pipette approximately 1 mL thawing medium back into the original vial and pour or pipette the remaining cells back into the 50 mL tube of thawing medium to ensure that all hepatocytes are transferred.
6. Suspend the cells by carefully rocking the 50 mL tube in your hands for a few seconds.
7. Centrifuge for 8 min at 100g.
8. Remove tube from centrifuge, disinfect and transfer to the BSC, and aspirate the supernatant to waste.
9. For every $1x10_6$ total cells expected, add ~1 mL of maintenance medium (for suspension hepatocytes) or ~0.8 mL plating medium (for palatable hepatocytes) to the cell pellet.
10. After this, depending upon the usage (i.e. suspension or plated applications), follow the instructions:

Procedure for Plated Use

11. ***Make sure you have your collagen coated plate ready before this step.***
12. Determine the viability and yield of your hepatocytes, using Trypan Blue exclusion method.
13. Add appropriate volume of plating medium (supplemented) to add to your current cell stock to achieve the desired cell density (use table below for recommended seeding density:

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Well type	Cell density (10^6/ml)
6 well	0.9-1.1
12 well	0.8-1.0
24 well	0.7-0.9
48 well	0.6-0.8

14. Place plate in a 37°C/5% CO_2 incubator. Incubate 4 – 6 hrs or until the cells are attached well.
15. Once the cells have attached, replace the plating media with maintenance media and incubate again in 37°C/5% CO_2 incubator for another 4-6 hours and thereafter replace
16. The hepatocytes are ready to use for further experiments
17. **Note:** *Mature human hepatocytes usually do not survive for longer than 7-10 days in culture. They do not proliferate and cannot be sub-cultured.*

Procedure for Suspension Use

18. Determine the viability and yield of your hepatocytes, using Trypan Blue exclusion method Add additional maintenance medium to bring cells to desired concentration of experimental design (most commonly 1x10_6 cells/mL).
19. It is recommended that you allow the hepatocytes to acclimate for 10 minutes by placing them on an orbital shaker at 120 rpm inside the incubator. Your hepatocytes are now ready to use.
20. **Note:** *Mature human hepatocytes usually do not survive for longer than 7-10 days in culture. They do not proliferate and cannot be sub-cultured.*

For laboratory research only. Not for clinical applications.
For technical questions, please email us at technical@abmgood.com

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