

Protocol: Plating Fresh Hepatocyte Suspensions

Note: Fresh hepatocyte suspensions from In Vitro Technologies do not need a Percoll enrichment step. The cells have >70% viability when measured by Trypan blue exclusion. Cells suspensions with a viability >70% are suitable for suspension studies or for plating. In Vitro Technologies cannot guarantee proper cell recovery if percoll enrichment is performed. Data obtained at In Vitro Technologies show that a Percoll enrichment step can cause a loss of 40 to 60% of the total cells.

In Vitro Technologies not guarantee that fresh hepatocytes obtained as suspension cultures from In Vitro Technologies will plate successfully. The only way to assure the best quality plated cells is to purchase them pre-plated from In Vitro Technologies.

Media Preparation:

1. Complete *InVitroGRO*TM HI Medium if running suspension assays
 - Thaw the *Torpedo* Antibiotic Mix in a 37° C water bath for 3 to 5 minutes.
 - Add 1.0 mL *Torpedo* Antibiotic Mix per 45 mL *InVitroGRO* HI medium.
 - Note: Following the addition of *Torpedo* Antibiotic Mix, the shelf life for the complete medium is 7 days.
2. Prepare Complete *InVitroGRO* CP Medium if plating hepatocytes.
 - Thaw the *Torpedo* Antibiotic Mix in a 37° C water bath for 3 to 5 minutes.
 - Add 1.0 mL *Torpedo* Antibiotic Mix per 45 mL *InVitroGRO* CP medium.
 - Note: Following the addition of *Torpedo* Antibiotic Mix, the shelf life for the complete medium is 7 days.

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1. Important: If suspensions cells will be plated, then use complete *InVitroGRO* CP medium for unpacking and recovering steps for fresh hepatocyte suspensions. If complete *InVitroGRO* HI medium is for unpacking and recovering fresh hepatocyte suspensions, the cells will not plate properly.
2. Follow the instructions for unpacking fresh hepatocyte suspensions.
3. After counting the cells, dilute the cells to $\sim 0.7 \times 10^6$ cells/ml in sterile, 37° C, isotonic, pH 7.4 complete *InVitroGRO* CP Medium.
4. Add an appropriate volume of diluted cells to collagen-coated tissue culture plates as follows:

6-well plate: 2.5 ml/well (requires a total volume of 15 ml per 6-well plate).

12-well plate: 1.0 ml/well (requires a total volume of 12 ml per 12-well plate).

24-well plate: 0.5 ml/well (requires a total volume of 12 ml per 24-well plate).

48-well plate: 0.2 ml/well (requires a total volume of 10 ml per 48-well plate).

96-well plate: add an equal volume of *InVitroGRO* CP medium (0.35×10^6 cells/ml), and add 100 μ L of this cell suspension to each well.

For T-flasks, add 0.25 ml/cm² to the T-flask (e.g., a T-75 flask receives 18.75 ml of cell suspension).

5. Gently shake the plates in a back-and-forth and side-to-side manner to evenly distribute the cells. Avoid any circular movement, as this will cause the cells to unevenly pool in the center of the plates.
6. Carefully place the plates into an incubator at 37° C, 5% CO₂, and saturating humidity to allow the cells to attach.

7. Allow the cells to incubate overnight or for at least 4 hours.
8. After the incubation period, remove a plate and examine the cells for attachment using a phase contrast microscope. Gently move the plate back and forth. The attached cells will move with the plate.
9. The cells should form a monolayer that is 70 to 100% confluent. This is a very subjective measurement based on the viewer's assessment of the percentage of the growth surface area that is covered by cells. The longer the cells are attached, the more they will become flat and shaped like irregular polygons; they will no longer be round and/or circular.
10. Gently aspirate the medium from the wells, and refill the wells of the plate with an appropriate volume of pre-warmed (37° C) complete *InVitroGRO* CP Medium.
11. Hepatocytes can be sandwiched using complete *InVitroGRO* CP Medium or complete *InVitroGRO* HI Medium.

References

1. Li, A. P. Primary hepatocyte cultures as an in vitro experimental model for the evaluation of pharmacokinetic drug-drug interactions. *Adv. Pharmacol. Series* **1997**, *43*, 103–130.

Caution: Treat all products containing human and monkey-derived materials a potentially infectious, as no known test methods can offer assurance that products derived from human or monkey tissues will not transmit infectious agents.

All products are for research use only. Do not use in animals or humans. These products have not been approved for any diagnostic or clinical procedures.