

Plateable Cryopreserved Hepatocytes

Product No.	Description	Size
M00995-P	Male Human	5 million viable cells*
F00995-P	Female Human	5 million viable cells*
F00005-P	Female Sprague–Dawley rat	5 million viable cells*
M00025-P	Male Wistar rat	5 million viable cells*
F00025-P	Female Wistar rat	5 million viable cells*
M00305-P	Male cynomolgus monkey	5 million viable cells*

*Check characterization table for available lots and sizes

Product Description

Hepatocytes are freshly isolated and cryopreserved on the same day. All plateable cryopreserved hepatocyte characterization information can be found by viewing the characterization tables at www.celsis.com/invitrotechnologies. Plateable cryopreserved hepatocytes are used for induction and toxicity studies¹. Our hepatocytes perform the best when used with Celsis In Vitro Technologies InVitroGRO hepatocyte media

Stability: Stable for at least 5 years at $\leq -150^{\circ}\text{C}$

Storage: $\leq -150^{\circ}\text{C}$

Thawing Procedure

Medium preparation

1. Prepare Complete *InVitroGRO* CP Medium
 - Place the *Torpedo* Antibiotic Mix in a 37°C water bath until thawed, then remove from water bath.
 - 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.

Thawing a single vial

1. Pre-warm *InVitroGRO* CP Medium to 37°C .
2. Transfer 5 ml of warm *InVitroGRO* CP Medium to a sterile 50 ml conical tube.
3. Carefully remove the vial from the shipping container or freezer. If the vial was stored in the liquid phase, carefully remove the cap and pour off any liquid nitrogen. Close the cap firmly before placing the vial into the water bath.
4. Immediately immerse the vial into a 37°C water bath. Shake gently until the ice is entirely melted, but no longer than it takes to completely thaw the vial. It may be helpful to remove the label from the vial so it is easier to view the vial contents.
5. Immediately empty the contents of the vial into the pre-warmed *InVitroGRO* CP Medium.
6. Add 1.0 ml of hepatocyte suspension to the vial to wash any remaining cells from the vial(s).
7. Resuspend the hepatocytes by gently inverting the tube several times (3 times is sufficient).
8. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.

9. Dilute cells to 0.70×10^6 viable cells/mL with *InVitroGRO* CP Medium.

Thawing multiple vials

Note: All vials should be thawed in the water bath simultaneously.

1. Pre-warm *InVitroGRO* CP Medium to 37° C. Ensure that there is enough medium for 5 mL of pre-warmed *InVitroGRO* CP Medium for each vial of cryopreserved hepatocytes. Use a container that will allow for re-suspending the cells.
2. After vials have thawed, quickly remove caps from each vial and pour the contents into a sterile tube or beaker that contains at least 5 mL of pre-warmed *InVitroGRO* CP Medium per vial thawed. For example, use 25 mL for 5 vials in a container that can hold a volume of 50 mL.
3. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.
4. Dilute the cells to 0.70×10^6 viable cells/mL with *InVitroGRO* CP Medium.

Procedure for Plating Cryopreserved Hepatocytes

Plating cryopreserved hepatocyte:

1. 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: 0.1 mL/well (requires a total volume of 10 mL per 96-Well plate)

For T-flasks, add 0.25 mL/cm² to the T-flask.

2. 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.
3. Carefully place the plates into a 37° C, 5% CO₂, saturating humidity incubator to allow the cells to attach.
4. Human hepatocytes will attach within 2-4 hours. However, to minimize handling time, they can be allowed to attach overnight.
5. **Rat and monkey hepatocytes will require an overnight incubation to achieve maximum attachment.**

Related Products

Product No.	Description	Size
Z99029	<i>InVitroGRO</i> TM CP (plating) medium	250 mL
Z990003	<i>InVitroGRO</i> TM CP (plating) medium	500 mL
Z990004	<i>InVitroGRO</i> TM CP (plating) medium	1 L
Z99000	<i>Torpedo</i> TM Antibiotic Mix	5.5 mL
Z990007	<i>Torpedo</i> TM Antibiotic Mix	11 ml
Z990008	<i>Torpedo</i> TM Antibiotic Mix	22 mL

Reference

1. Roymans, D.; Van Looveren, C.; Leone, A.; Parker, J. B.; McMillan, M.; Johnson, M. D.; Koganti, A.; Gilissen, R.; Silber, P.; Mannens, G.; Meuldermans, W. Determination of cytochrome P450 1A2 and cytochrome P450 3A4 induction in cryopreserved human hepatocytes. *Biochem. Pharmacol.* **2004**, *67*(3), 427-437.

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.