

DonorPlex™ Hepatocytes: Suspension Format

Technical information & instructions

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I. Introduction

This protocol is suitable for the thawing cryopreserved hepatocytes. Please read through this entire protocol before attempting this procedure. The health of the hepatocytes is dependent upon following the protocol carefully. For all cryopreserved hepatocytes, a thawing medium is required to ensure good viability following thaw. Suspension qualified DonorPlex™ Hepatocytes are non-adherent, and non-proliferative and cannot be passaged. It's important to follow the counting and seeding guidelines to make sure your hepatocytes function properly for the duration of your experiments.

For answers to frequently asked questions and citations regarding these products, please visit our knowledge center:

<https://knowledge.lonza.com>

II. Required reagents and materials

(components sold separately)

- DonorPlex™ Pooled Donor Hepatocytes
- Thawing medium: Catalog # MCHT50P
- 37°C degree water bath
- Biological Safety Cabinet (BSC)
- Room temperature centrifuge capable of spinning 50mL conical tubes at 65-200 x g
- 120 rpm orbital shaker inside a cell culture incubator
- 37C/5% CO₂ Incubator

Consumables:

- Wide bore pipets and pipet tips
- Automated pipettor and serological pipet
- 0.4% solution of Trypan Blue

Using media or reagents other than what is recommended will void the cell warranty (See Section VIII). Please contact Scientific Support if you need help selecting media and/or reagents.

III. General cell information

	Species	Cat. no.	Description	Characterization (see website for more details)
Formats	Human	HUCS10P	Human Hepatocytes, 10-Donors Pooled Susp. Qualified, Mixed Gender	<p>All DonorPlex™ Hepatocyte lots are characterized for the following attributes and functions:</p> <p>Suspension metabolism detecting CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 using probe substrates and measurement using mass spectrometry</p> <p>Suspension ECOD analysis for general metabolism, SULT, and UGT activities measures using 7-ethoxycoumarin substrate and measurement of metabolites by mass spectrometry</p> <p>Transporter function detecting activity for QAT1B1/3, OCT1, and NTCP using a facilitated active uptake assay</p> <p>Post-thaw Yield</p> <p>Post-thaw Viability</p>
		HUCS20P	Human Hepatocytes, 20-Donors Pooled Susp. Qualified, Mixed Gender	
		HUCS10PF	Human Hepatocytes, 10-Donors Pooled Susp. Qualified, Female	
		HUCS10PM	Human Hepatocytes, 10-Donors Pooled Susp. Qualified, Male	
		HUCS20PF	Human Hepatocytes, 10-Donors Pooled Susp. Qualified, Female	
		HUCS20PM	Human Hepatocytes, 20-Donors Pooled Susp. Qualified, Male	
		HUCS50P	Human Hepatocytes, 50-Donors Pooled Susp. Qualified, Mixed Gender	

IV. Unpacking and storage instructions

- For cryopreserved cells:** Remove cryovials from the liquid nitrogen shipping dewar and immediately place into vapor phase liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If shipping dewar is warm, please contact Customer Service.
- For MCHT50P:** Unpack the 50 mL conical tubes containing 40-45 mLs of thawing medium and store upright at 2°- 8°C. Use by expiration date listed on the label.

V. Thawing of Cells

- Warm MCHT50P thawing media in a 37°C water bath. Once the thawing medium is warmed, disinfect it (70% ethanol wipe or spray) and transfer it to the BSC.

NOTE: The next 6 steps should occur as quickly as possible and without distraction

- Remove the cryopreserved hepatocytes from their storage location (shipping dewar or storage

dewar) and quickly submerge as much of the vial as possible, up to the cap, in the water bath. It is important to make sure the cap of the vial stays above the waterline.

- Thaw the vial for approximately 90–120 seconds. The vial will thaw from the outside to the inside. You can see a spindle form and shrink as the vial thaws.
- When almost completely thawed and only a small spindle of frozen cells remains, remove vial from water bath, disinfect the vial and transfer it to the BSC.
- Quickly remove vial cap and carefully pour or pipette (with a wide-bore tip) hepatocytes into the 50 mL conical tube of appropriate warmed thawing medium.
- 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.
- Suspend the cells by carefully rocking the 50 mL tube by hand, for a few seconds. **DO NOT VORTEX.**

8. Centrifuge 200xg for 10 minutes at room temperature
9. Remove tube from centrifuge, disinfect, and transfer to the BSC.
10. Pour supernatant into a waste bottle, inverting completely, without shaking (or aspirate off supernatant carefully with a vacuum aspirator).
11. For each vial, gently resuspend cells in warm 3 mL serum-free medium per experimental needs.
12. Determine the viability and yield of your hepatocytes using the Trypan Blue exclusion method (See section VII Cell Counting Procedure for assistance).

VI. Procedure for cell counting

To determine cell viability and viable cell yield with the Trypan Blue Exclusion Method for Hepatocytes, follow the directions below. Trypan Blue Exclusion Method must be used to accurately determine viability and yield of hepatocytes. Use of any other method may result in viability and yield different from that shown on the lot specific CoFA.

1. To a clean microfuge tube, add 50 μ L of 0.4% Trypan Blue Solution, 350 μ L of medium and 100 μ L of cell suspension. Following this example results in a 1:5 fold dilution of your hepatocytes. If a different dilution is desired, volumes may be adjusted as long as the 0.4% Trypan Blue Solution still represents no more than 10% of the total volume (final Trypan blue is 0.04%).
2. Determine cell viability using the formula below.

$$\text{Eq. 1: } 100 \times (\text{Live cell count} \div \text{Total cell count}) = \text{Viability\%}$$

3. Determine total viable cell yield using the formula below.

$$\text{Eq. 2: } \text{Viable cell count} \div \text{Quadrants counted} \times \text{Dilution factor} \times 10000 \times \text{Current volume (mL)} = \text{Viable cell yield}$$

Example: 100 cells \div 4 quadrants \times 5 \times 10000 \times 3mL total volume = 3,750,000 cells

VII. Initiation of culture

1. Add additional maintenance medium or your experimental buffer per specific user application to bring cells to desired concentration of experimental design (most commonly, 1×10^6 cells/mL).
2. It is recommended that you allow the hepatocytes to acclimate for 10 minutes by placing them on an orbital shaker located inside the incubator at 120 rpm. Your hepatocytes are now ready to use.

VIII. Product warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of cells only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

When placing an order or for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Primary Cell Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or want to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone or mail. (See Page 1 for details).

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

WARNING: LONZA PRIMARY CELL PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, HIV-2, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, HIV-2, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, HIV-2, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5thed. If you require further information, please contact your site safety officer or Scientific Support.

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