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Product Information

Primary Human Hepatocytes
LifeNet Health®

Catalog Number:
MTOXH1000 - Cryoplateable Primary Human Hepatocytes
MTOXH1001 - Cryoplateable Primary Human Hepatocytes Short-term
MTOXH1002 - Cryoplateable Primary Human Hepatocytes Induction Certified
MTOXH1003 – Cryoplateable Primary Human Hepatocytes Long-term
MTOXH1005 - Cryopreserved Primary Human Hepatocytes Suspension

Storage Temperature for Hepatocytes is -135 °C to -190 °C in the vapor phase of liquid nitrogen

MTOXH1008 - Cryoplateable Primary Human Hepatocytes NASH
MTOXH1009 - Cryopreserved Primary Human Hepatocytes NASH
MTOXH1011 - Cryopreserved Primary Human Hepatocytes Neonatal
MTOXH1012 - Cryopreserved Primary Human Hepatocytes Juvenile

MED-HHTM - Human Hepatocyte Thawing Medium
MED-HHPM - Human Hepatocyte Plating Medium
MED-HHCM - Human Hepatocyte Culture Medium
MED-HHPMS -Human Hepatocyte Plating Medium Supplement
MED-HHCMS -Human Hepatocyte Culture Medium Supplement

TECHNICAL BULLETIN

Product Description
Primary human hepatocytes from LifeNet Health meet the specific needs of a wide range of scientific research applications. Donated liver tissues are procured under state-of-the-art conditions using the highest standards for tissue recovery and preservation, which utilize enhanced tissue handling and transportation methods, and minimize warm and cold ischemia time to optimize tissue processing outcomes. These measures, combined with refined cell isolation techniques and advanced post-thaw characterization, represent a new industry standard for hepatocyte quality and performance.

Prior to release, each batch of cryopreserved hepatocytes is fully characterized to determine the post-thaw results. The batch-specific functionality and donor data includes the following:

- Cell viability and yield per vial
- Morphological integrity and attachment efficiency
- Optimal seeding density
- Enzyme activity
- Cytochrome P450 (CYP) induction response (when applicable)

A Certificate of Analysis (CoA) is available for each batch and includes comprehensive donor history, histological images with pathology results, post-thaw characterization data, and respective cell culture images.

For optimal cell performance, LifeNet Health Human Hepatocyte Thawing (HHTM), Plating (HHPM), and Culture (HHCM) Media are recommended. LifeNet Health's HHTM is formulated to maximize the yield and viability of hepatocytes following cryopreservation. HHPM is optimized for attachment of hepatocytes to cultureware. HHCM is serum-free and maintains healthy hepatocytes during cultivation.

Component
This product is a cryovial containing approximately 5 million primary human hepatocytes. Refer to the CoA of the specific lot for the actual cell yield.

Reagents and Equipment Required but Not Provided.

Media and Supplements
Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- Human Hepatocyte Thawing Medium (HHTM) (Catalog Number MED-HHTM)
- Human Hepatocyte Plating Medium (HHPM) (Catalog Number MED-HHPM)
- Human Hepatocyte Culture Medium (HHCM) (Catalog Number MED-HHCM)
- Human Hepatocyte Plating Medium Supplement (Catalog Number MED-HHPMS)
- Human Hepatocyte Culture Medium Supplement (Catalog Number MED-HHCMS)

Receiving

- Cryogenic storage freezer
- Small laboratory ice tray
- Tongs or forceps
- Liquid nitrogen
- Cryovial storage box

Thawing and Plating

- Biological safety cabinet
- 70% ethanol (prepared from Ethanol, Catalog Number E7148)
- Bio-Pure™ alcohol wipes (Catalog Number Z688487)
- Small laboratory ice tray
- Portable Dewar or other container to transport frozen vials in liquid nitrogen
- Tongs or forceps
- Ice
- Liquid nitrogen
- Cryovial storage box
- 37 °C water bath (operating range 35–38 °C)
- Centrifuge capable of achieving 100 × *g* with 50 mL conical tube adaptors
- 1,000 µL multichannel electronic pipette
- 1,000 µL multichannel electronic pipette tips
- Serological pipettor with 1, 2, 5, 10, and 25 mL sterile pipettes
- Vacuum aspiration system and sterile plastic or glass aspiration tips (optional)
- Sterile 50 or 100 mL reagent reservoir
- Collagen coated (rat tail, Type I) culture multiwell plates
- Disposable plastic or washable glass media bottles (100–250 mL)
- Timer
- Sterile 50 mL conical tubes
- 20, 200, and 1,000 µL micropipettes
- Sterile 20, 200, and 1,000 µL pipette tips
- 0.2 µm filter units (optional)
- 37 °C / 5% CO_2 incubator (humidified)
- 0.4% Trypan Blue solution (Catalog Number T8154)
- Acridine orange/propidium iodide (AO/PI) stain for cell counting (optional)
- Hemocytometer or equivalent cell counting device
- Antibiotic (optional), e.g. Penicillin/Streptomycin is recommended (Catalog Number P0781)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Potential Biohazard. Handle as if capable of transmitting infectious agents.

Procedures

NOTE: Read and understand the following instructions prior to use. Improper handling may adversely affect cell quality and performance.

Receiving Cryopreserved Human Hepatocytes

1. Transport the cryogenic shipping container holding the cryopreserved hepatocyte vial(s) next to the cryogenic storage freezer.
2. Wear personal protective equipment (PPE) appropriate for liquid nitrogen and cryovial handling.
3. Fill an insulated laboratory ice tray with enough liquid nitrogen to submerge only a few millimeters of a cryovial storage box.
4. Unseal the shipping container by opening the flaps and removing the cap/plug of the inner Dewar container.

   Note: A small amount of vapor may rise from the Dewar container.

5. Open the cryogenic storage freezer and remove the appropriate rack and box for storing the received vials. Place the storage box in the liquid nitrogen-containing tray.
   - If the vials were shipped in a cryovial storage bag, proceed to Step 6.
   - If the vials were shipped in a cryovial storage box, proceed to Step 9.

6. Quickly lift the shipping bag from the cryogenic shipping container using the handles provided.
7. Immediately open the bag and use forceps or tongs to transfer the vials to the cryovial storage box in the liquid nitrogen-containing tray.
8. Quickly replace the storage box into the rack of the cryogenic storage freezer.
9. When receiving larger numbers of vials, they may be shipped in cryovial storage boxes. Either place this box quickly into an empty slot in the cryogenic freezer rack or place the shipping box into the liquid nitrogen-containing tray next to the cryovial storage box. Use forceps or tongs to transfer the vials from the shipping box to the storage box.
10. Repeat Steps 6-8 or Step 9 as applicable, until all vials have been transferred to the cryogenic storage freezer.
11. Follow the instructions provided with the cryogenic shipping container for return shipment.

Preparation of Human Hepatocyte Media (HHTM, HHPM, and HHCM)

Note: At least one 50 mL bottle of Human Hepatocyte Thawing Medium (Catalog Number MED-HHTM) is needed per vial of cryopreserved human hepatocytes. Up to three (3) vials of cryopreserved human hepatocytes (maximum of 40-50 $\times$ 10^6 cells) can be placed in one 50 mL conical tube with Human Hepatocyte Thawing Medium. DO NOT overload container of Human Hepatocyte Thawing Medium with excess cells.

Note: Do NOT warm media for an excessive period of time.

Note: Supplemented HHPM and HHCM media should be prepared fresh daily for best results. If not prepared daily, use within 72 hours of preparation.

1. Remove Human Hepatocyte Thawing Medium (HHTM) bottle(s) (Catalog Number MED-HHTM) from –20 °C storage and thaw immediately prior to use in a 37 °C water bath protected from bright light for approximately 20-30 minutes.
   Note: One bottle of HHTM can be used for up to three (3) vials of cryopreserved human hepatocytes (maximum of 40-50 $\times$ 10^6 cells).
2. Remove Human Hepatocyte Plating Medium Supplement (Catalog Number MED-HHPMS) and Human Hepatocyte Culture Medium Supplement (Catalog Number MED-HHCMS) from –20 °C storage and thaw immediately prior to use in a 37 °C water bath.
3. Remove Human Hepatocyte Plating Medium (HHPM) (Catalog Number MED-HHPM) and Human Hepatocyte Culture Medium (HHCM) (Catalog Number MED-HHCM) from 4 °C storage.
4. Working in a biological safety cabinet, add 15 mL of thawed Human Hepatocyte Plating Medium Supplement to the HHPM Medium. Mix well by gently inverting the container several times.

   Note: If desired, an antibiotic can be added to the Supplemented HHPM. Penicillin/Streptomycin at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.

5. Optional step: Filter Supplemented HHPM through 0.2 µm filter.
6. Working in a biological safety cabinet, add 5 mL of thawed Human Hepatocyte Culture Medium Supplement to the HHCM Medium. Mix well by gently inverting the container several times.

   Note: If desired, an antibiotic can be added to the Supplemented HHCM. Penicillin/Streptomycin at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.

7. Optional step: Filter Supplemented HHCM through 0.2 µm filter.
8. Aliquot a minimum of 20 mL of Supplemented HHPM into a sterile 50 mL conical centrifuge tube. Place in a 37 °C water bath for approximately 20-30 minutes. Protect from bright light and do NOT warm for excessive periods of time.
9. Aliquot the needed amount of Supplemented HHCM into a sterile conical centrifuge tube or bottle depending on volume required. Hold the aliquot at room temperature. Protect from bright light until ready to use. Store excess Supplemented HHCM at 4 °C.

Thawing Cryopreserved Human Hepatocytes

1. Refer to the batch specific CoA for average yield per vial as well as any special centrifugation instructions before proceeding with the following steps.
2. Wear personal protective equipment (PPE) appropriate for liquid nitrogen and human hepatocyte handling.
3. Fill a portable Dewar flask or other liquid nitrogen container with a small amount of liquid nitrogen (enough to submerge a cryovial to at least half of its height).
4. Set up the following in a biological safety cabinet:
    - 50 mL sterile conical centrifuge tube(s)
    - 1,000 µL pipette with 1,000 µL sterile tips
    - Small laboratory ice tray containing ice
    - Aspiration tips
    - Serological pipettor with various sterile serological pipette tips
5. Remove bottle(s) of HHTM from 37 ℃ water bath, spray with 70% ethanol, and place in biological safety cabinet. Do NOT invert/mix the HHTM.
6. Transfer HHTM from each bottle with a serological pipette to a sterile 50 mL conical centrifuge tube.
    - If thawing single vial of hepatocytes, transfer 49 mL of HHTM to the tube.
    - If thawing more than one vial of hepatocytes, transfer 1 mL less of HHTM per vial being thawed, up to 3 mL (three vials) per tube.
7. Cap the filled 50 mL conical centrifuge tube(s) and gently invert three times to mix HHTM thoroughly.
8. Optional step: Filter HHTM through 0.2 µm filter after aliquoting into the tube(s).
9. Place small insulated laboratory ice tray containing ice next to the 37 °C water bath.
10. QUICKLY remove vial(s) with cryopreserved human hepatocytes from cryogenic storage freezer and place in the portable Dewar flask or liquid nitrogen container.
11. Carefully transfer cryopreserved cells in liquid nitrogen container to culture area.

    Note: Transferring vials from the liquid nitrogen, loosening caps, and submerging into water (Steps 12 and 13) should take no more than 10 seconds in order to maintain hepatocyte viability and functionality.

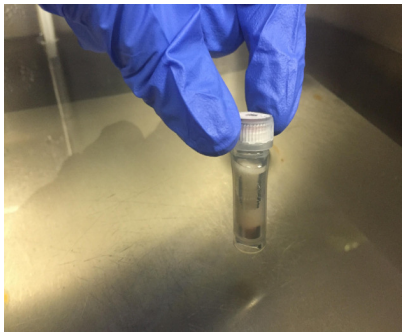
12. Using tongs or forceps, QUICKLY remove vial(s) of cryopreserved human hepatocytes from liquid nitrogen and loosen vial cap(s) slightly to release pressure, then re-tighten cap(s).

    Note: Do NOT remove cap(s) or expose the contents to air!

13. Partially submerge vial(s) vertically into 37 °C water bath, to ~0.5 cm below the cap (see Figure 1). Ensure the water level is at least even with the top of the frozen cell suspension. Thaw the vial for 90-100 seconds using a timer.

    Note Timing of the thaw step is critical. It is important to use a timer for Steps 13-14.

Figure 1.
Correct placement of vial in water bath for thawing



Note: The vial is submerged to the top of the frozen cell suspension; however, the cap is above the water.

14. Remove vial(s) from 37 °C water bath and invert. If the frozen cell suspension has begun to liquify and slides freely without tapping or shaking the vial(s), return the vial(s) to upright and continue to Step 15. If the frozen cell suspension does not slide freely, return the vial(s) to upright and place back in the water bath for an additional 5 seconds. Recheck the movement of the cell suspension before continuing to Step 15. Repeat as necessary if frozen cell suspension continues to not move freely.

    Note: Place sufficiently thawed vials on ice while waiting for remaining vials to thaw.

15. QUICKLY place vial(s) in ice tray for transport and clean cap and neck of each vial with alcohol wipe prior to placement in the biological safety cabinet.
16. Moving at a quick pace and working with one vial at a time, remove vial cap and pour hepatocytes into the prepared 50 mL conical tube of HHTM.

17. Using 1,000 µL pipette, rinse vial with 1,000 µL of the HHTM-cell suspension and then transfer the rinse back into the 50 mL tube of HHTM. Repeat this step for all other vials (working quickly).
18. Tighten cap(s) on tube(s) and gently invert tube(s) three times to uniformly suspend hepatocytes.
19. Centrifuge hepatocytes at 100 × *g* for 8 minutes at room temperature.

    Note: Cells from individuals with a high BMI may have slightly different centrifugation conditions. Examine the CoA for the specific batch for any special instructions prior to centrifugation in order to maximize the yield.

20. Return the tube(s) to the biosafety cabinet and gently vacuum aspirate the supernatant without disturbing the cell pellet at the bottom of the tube.
21. Add 3–5 mL of warm Supplemented HHPM or Supplemented HHCM to the tube(s). Volume of media depends on the expected cell yield. Use appropriate type of media (plating or culture) for the subsequent experiment.
22. Gently rock the conical tube to resuspend hepatocytes. Do NOT resuspend cells by swirling vigorously or by using a pipette or vortex mixer.

    Note: Typical concentration for cell counting should be 1–2 × 10^6 cells/mL to allow for accurate cell counting. Average yield per vial is included in the CoA for each batch.

23. Count hepatocyte suspension to determine cell yield and viability by using the Trypan Blue exclusion test on a hemocytometer and/or AO/PI staining with an automated cell counter.

    Note: Use a 10-fold dilution for the trypan blue exclusion method or a 2-fold dilution for AO/PI staining.

    Note: Accurate concentration of the cell suspension is critical for proper plating. Use a manual counting method if a hepatocyte-specific program is not available.

24. Proceed to plating cells or using the cells for suspension assays.

Plating Cryopreserved Human Hepatocytes

1. After thawing the cryopreserved human hepatocytes refer to the CoA for each batch of hepatocytes for recommended seeding density for standard collagen-coated 24-well plates.

   Note: The seeding density is unique to each batch for cryopreserved human hepatocytes. A range of densities is provided in Table 1, but the actual final density for a specific batch is provided in the CoA. If not using 24-well plates, determine the appropriate cell density per batch by testing different densities (refer to Table 1 for ranges).

2. Determine the final cell density and volume required for application. Using the final cell yield determined in Step 22 above, add warm Supplemented HHPM to cell suspension until desired final cell density is reached. If the final volume required is greater than 50 mL, transfer the cell suspension from the 50 mL conical tube to an appropriately sized sterile bottle.
3. Ensure the suspension is homogeneously mixed by gentle rocking or inverting.
4. Pour cell suspension into the sterile reagent reservoir until half full.
5. Using a multichannel pipette, transfer cell suspension from reagent reservoir to culture plate(s).

   Note: Hepatocytes settle out of suspension quickly. Gently rock the reservoir in all directions to ensure the cell suspension is homogenous when refilling pipette. Similarly, gently mix the cell suspension by inverting the conical tube or bottle prior to refilling the reservoir.

6. Remove culture plate(s) from the biological safety cabinet in stacks of 1-3 and place in a humidified incubator (37 °C, 5% CO_2).
7. Shake/rock culture vessels in a north/south then east/west manner, followed by a figure 8 movement, three times on the incubator shelf. This shaking should be gentle yet vigorous enough to displace cells grouped in the center of each well. Repeat the north/south and east/west motion at 15 minute intervals for the first 60 minutes of culture. Shake the plates again at 90 minutes of culture.

   Note: 96-well plates do NOT need to be shaken prior to placement in the incubator nor at 15 minute intervals for the first 60 minutes of culture.

Culturing Thawed Cryopreserved Human Hepatocytes

1. At 2–4 hours post plating (dependent on cell attachment), remove culture plate(s) from the incubator and place in the biological safety cabinet. Do not move more than 3 culture plates at one time.
2. Warm only the necessary amount of Supplemented HHCM to 37 °C in a water bath protected from bright light for 20-30 minutes prior to use. See Table 2 for recommended volumes.
3. Add the needed amount of warmed Supplemented HHCM into a sterile reagent reservoir.
4. Gently shake plates in a north/south then east/west manner on a solid surface, to dislodge dead cells.
5. Tilt plates and gently vacuum aspirate the medium from the side of each well without touching the cell monolayer.

   Note: Avoid prolonged or excessive aspiration of wells which may cause dehydration of the cells.

6. Using a multichannel pipette add warm Supplemented HHCM in volumes shown in Table 2.
7. Return plate(s) to incubator for overnight or until experimental protocol dictates.
8. If warranted, overlay cells with extracellular matrix 8-10 hours post-plating.
9. Replace medium daily with fresh warm Supplemented HHCM.

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Table 1.
Seeding Densities for Cryopreserved Human Hepatocytes

Culture Vessel	Seeding Density (cells × 10^6/mL)	Volume per Well
6 well plate	0.75–1.1	2 mL
12 well plate	0.8–1.0	1 mL
24 well plate	0.7–1.0	500 µL
48 well plate	0.6–0.8	200 µL
96 well plate	0.4–0.6	150 µL

Table 2.
Volumes of Supplemented Human Hepatocyte Culture Medium for Plating

Culture Vessel	Volume per Well
6 well plate	1.5 mL
12 well plate	1 mL
24 well plate	500 µL
48 well plate	200 µL
96 well plate	100 µL