FOR NON-CLINICAL RESEARCH USE ONLY

Product Description

LifeNet Health's TruVivo Flex Kit consists of primary human feeder cells and glucocorticoid-free media components. All cells are isolated from donated human tissue, resulting from the generous gift of an individual or their family.

Indications for Use

For research use only. Not for use in diagnostic procedures nor for implantation into humans.

Warnings and Precautions

Observe universal precautions when handling human-derived tissues and cells as they are potentially biohazardous. Refer to the guidelines set forth in Occupational Safety and Health Standards for handling blood, tissues, body fluids, or other potentially infectious materials. Follow institutional guidelines for the collection and disposal of all solid and liquid waste that has been in contact with these products.

Transfer or resale of any LifeNet Health cells or products is prohibited without the written consent of LifeNet Health.

Donor Screening and Testing

Donor authorization for non-clinical research use of these cells was appropriately obtained and documented by LifeNet Health. All donors are tested and confirmed negative for the following infectious diseases: Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Syphilis, Toxoplasmosis, and beginning March 2020 SARS-CoV-2 (COVID-19).

Storage Requirements

The distributor, intermediary and/or end-user is responsible for storing TruVivo components under appropriate conditions prior to further distribution or use. LifeNet Health ships frozen cells in the vapor phase of liquid nitrogen (-135°C to -190°C). On receipt, immediately transfer frozen cells to storage in the vapor phase of liquid nitrogen (-135°C to -190°C) until ready for experimental use. Do not thaw and refreeze. LifeNet Health ships media either on dry ice (-10°C to -30°C) or on refrigerated cold packs (2°C to 8°C) depending on the appropriate storage condition. On receipt, immediately transfer media to the appropriate storage condition as indicated on the product label until ready for experimental use.

Final Product Testing

Each feeder cell lot is qualified for use in the TruVivo system. Hepatocytes derived from Sprague-Dawley and Wistar rats have been shown to exhibit consistent performance in the system meeting minimum specifications for plateability, monolayer confluency, morphology, albumin, urea secretion, and induction of P450 CYP3A23 over a 14-day culture period.

Complaints and Returns

For further information or returns or to report a complaint, please contact LifeNet Health Client Services (available 24 hours a day) at 1-888-847-7831 (inside the U.S.) or 00+1-757-464-4761 ext. 2000 (outside the U.S.) and have the product code and lot number available (see COA or Order Confirmation).

TruVivo Flex Kit for Rat Hepatocytes

It is important to read and understand the following instructions prior to use. Improper handling may adversely affect cell quality and performance.

Before handling any materials, don appropriate personal protective equipment (PPE) for liquid nitrogen (LN_2) and cryovial handling.

I. Receiving and Storage

Cryopreserved Cells:

1. Transport the shipping container containing the cryopreserved feeder cell vials next to the cryogenic storage freezer.
2. Fill insulated laboratory ice tray with enough LN_2 to submerge only a few millimeters of a cryovial storage box.
3. Unseal the shipping container by opening the flaps and removing the cap/plug of the inner dewar. **NOTE:** You may notice a small amount of vapor rising from the dewar.
4. Open the cryogenic storage freezer and remove the appropriate rack and box for storing the received vials. Place the storage box in the LN_2-containing tray.
5. Using forceps or tongs, transfer the vials from the shipping box to the storage box.
6. Repeat Step 5 until all vials have been transferred to the cryogenic storage freezer.
7. Follow the instructions provided with the shipping dewar for disposal or return shipment.

Frozen Medium (Green Labeled Bag):

1. Quickly transfer bag containing Feeder Cell Thawing Medium (FCTM) and Supplements B, D, and E from shipping box to a -20°C freezer until ready to use. **NOTE:** All media should be protected from light.

Refrigerated Medium (Orange Labeled Bag):

1. Quickly transfer bag containing TruVivo Plating Medium (TCPM) and TruVivo Culture Medium (TCCM) from shipping box to 4°C refrigerator until ready to use. **NOTE:** All media should be protected from light.

II. Week 1 Medium Preparation

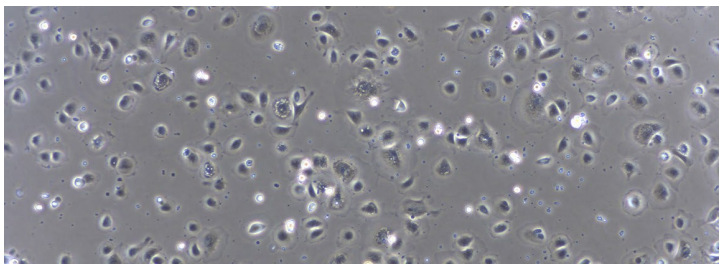
1. One day before use, transfer FCTM and Supplements B, D, and E from -20°C to 4°C storage and thaw overnight. **OPTIONAL:** Thaw at room temperature or 37°C prior to use.
2. Spray and wipe all reagent bottles (FCTM, TCPM, TCCM, and Supplements B, D, and E) with 70% (v/v) alcohol and place in biological safety cabinet (BSC).
3. Prepare TCPM by adding 4.5 mL Supplement B and 11 mL Supplement D to the bottle of TCPM. TCPM contains the needed concentration of Dexamethasone (Dex) for cell plating. **NOTE:** If desired, an antibiotic can be added to TCPM. Penicillin/Streptomycin, at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.
4. For the 24-well culture plate format, prepare TCCM by aliquoting 100 mL of TCCM and adding 14 mL of Supplement D and 1 mL of Supplement E. For the 96-well culture plate format, aliquot 150 mL of TCCM and add 22 mL Supplement D and 1.5 mL Supplement E. Dex also needs to be added to achieve the rat-specific final concentration of 10 nM. **NOTE:** If desired, an antibiotic can be added to TCCM. Penicillin/Streptomycin, at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.
5. Transfer FCTM into a sterile 15 mL conical tube and gently invert 3 times to mix thoroughly.
6. Prior to use, warm reagents in 37°C water bath for 20-30 minutes.
7. Following addition of supplements to basal mediums, re-freeze Supplements D and E in a -20°C freezer until week 2 of culture.

III. Thawing and Plating Human Feeder Cells

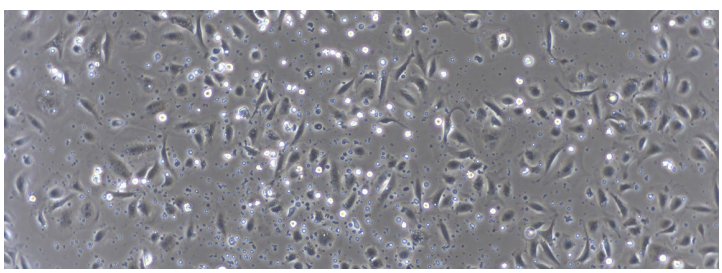
NOTE: Rat hepatocytes should be thawed approximately 60 minutes following completion of this section.

1. Remove 15 mL conical tube of FCTM from 37°C water bath, spray, and wipe with 70% (v/v) alcohol, and place in BSC.
2. Wear appropriate PPE for handling liquid nitrogen (LN_2) and biohazardous materials (Refer to OSHA Standard 29CFR1910.132).
3. Fill a suitable container with enough LN_2 to submerge a cryovial to at least half of its height.
4. Place a small, insulated laboratory ice tray filled with ice, for transporting cells, next to 37°C water bath.
5. Quickly remove cryovial of feeder cells from LN_2 storage and place in the portable LN_2 container.
6. Carefully transport frozen cryovial in the LN_2 container to the 37°C water bath.
7. Using forceps or tongs, QUICKLY remove cryovial of feeder cells from the LN_2, loosen the cryovial cap slightly to release pressure and then re-tighten. **NOTE:** Do NOT completely remove the cryovial cap.
8. Partially submerge cryovial vertically in 37°C water bath to thaw for 90 seconds. Ensure the water level is at least even with the top of the frozen cell suspension, being careful to keep the neck of the cryovial above the water.
9. Remove cryovial from 37°C water bath and gently invert. The cell suspension should be somewhat liquefied and slide freely without tapping or shaking the cryovial. If the frozen suspension does not slide freely, return cryovial to upright position and place back in water bath for an additional 5 seconds. Recheck the movement of the cell suspension.
10. Quickly place in an ice tray to prevent the cells from reaching room temperature. Transport to the BSC. Wipe the cryovial with 70% (v/v) alcohol prior to placing in BSC.
11. Transfer thawed feeder cells to 15 mL tube containing FCTM.
12. Using a 1000 µL pipette, rinse the cryovial once with approximately 1000 µL of FCTM from the tube. Transfer the FCTM back to the tube.
13. Gently pipette 2-3 times to uniformly resuspend the feeder cells.
14. Centrifuge feeder cells at 400 x g for 4 minutes at room temperature.
15. Return to BSC and gently aspirate the supernatant without disturbing the cell pellet in the bottom of the tube.
16. Resuspend the cell pellet in 1 mL of warm supplemented TCPM taking care to not create bubbles.
17. Count the feeder cell suspension by either the trypan blue exclusion method on a hemocytometer and/or AO/PI staining with an automated cell counter to determine yield and viability. **NOTE:** Use a 10-fold dilution for the trypan blue exclusion method or a 2-fold dilution for the AO/PI staining.
18. Add additional warm supplemented TCPM to cell suspension to dilute the feeder cell suspension to 100,000 cells/mL.
19. Pipette the entire feeder cell suspension into a sterile reagent reservoir.
20. Using a multi-channel pipette, transfer 500 µL of cell suspension (50,000 cells) from the reservoir to each well of a Collagen I-coated 24-well plate or 100 µL of cell suspension (10,000 cells) to each well of a Collagen I-coated 96-well plate. Gently shake the reservoir in a N-S, E-W orientation to ensure the cell suspension is homogeneous prior to each refill of the pipette.
21. Remove culture plate from the BSC and place in a humidified incubator (37°C, 5% CO_2).
22. For the 24-well format, gently shake the culture plate in a N-S, E-W orientation 5 times for even distribution of feeder cells. Do NOT shake the 96-well format.
23. Visually inspect feeder cell attachment 60 minutes post-plating, prior to start of hepatocyte handling. Feeder cell attachment should be similar to the images provided below.

Figure 1. Feeder Cell attachment



TruVivo Human Feeder Cells 60 minutes post-plating



TruVivo Human Feeder Cells 90 minutes post-plating

IV. Thawing and Plating Rat Hepatocytes

NOTE: Proceed with this section once feeder cells have successfully attached.

1. Remove 50 mL conical tube of manufacturer recommended thawing medium from 37°C water bath, spray, and wipe with 70% (v/v) alcohol, and place in BSC.
2. Refill portable LN_2 container with enough LN_2 to submerge a cryovial to at least half of its height.
3. Place small, insulated laboratory ice tray filled with ice, for transporting cells, next to 37°C water bath.
4. Quickly remove cryovial of rat hepatocytes from LN_2 storage and place in the portable LN_2 container.
5. Carefully transport frozen cryovial in the LN_2 container to the 37°C water bath.
6. Using forceps or tongs, QUICKLY remove cryovial of rat hepatocytes from the LN_2, loosen the cryovial cap slightly to release pressure and then re-tighten. **NOTE:** Do NOT completely remove the cryovial cap.
7. Partially submerge cryovial vertically in 37°C water bath to thaw for 90 seconds. Ensure the water level is at least even with the top of the frozen cell suspension, being careful to keep the neck of the cryovial above the water.
8. Remove cryovial from 37°C water bath and invert. The cell suspension should be somewhat liquefied and slide freely without tapping or shaking the vial. If frozen cell suspension does not slide freely, return cryovial to upright position and place back in water bath for an additional 5 seconds. Recheck the movement of the cell suspension.
9. Quickly place cryovial in ice tray to prevent the cells from reaching room temperature, and transport to BSC. Wipe the cryovial with 70% (v/v) alcohol prior to placing in BSC.
10. Remove the cryovial cap and pour hepatocytes into tube containing thawing medium. Using a 1000 µL pipette, rinse the cryovial once with approximately 1000 µL of suspension from the tube and collect.
11. Tighten the tube cap and gently invert the tube 3 times to uniformly resuspend the hepatocytes.
12. Centrifuge the hepatocytes at the speed, duration of time, and temperature recommended by the manufacturer.
13. Return to BSC and gently aspirate the supernatant without disturbing the cell pellet in the bottom of the tube.
14. Add 3 mL of warm supplemented TCPM to hepatocytes. Gently rock the tube to resuspend the hepatocytes. **NOTE:** Do NOT pipette or vortex to mix or swirl vigorously.
15. Add 5 mL of additional warm supplemented TCPM to hepatocytes and count the suspension by either trypan blue exclusion method on a hemocytometer and/or AO/PI staining with an automated cell counter to determine yield and viability. **NOTE:** Use a 10-fold dilution for the trypan blue exclusion method or a 2-fold dilution for the AO/PI staining.
16. Add additional warm supplemented TCPM to cell suspension to dilute hepatocytes to 600,000 cells/mL for the 24-well format or 300,000 cells/mL for the 96-well format.
17. Immediately prior to plating hepatocytes, remove culture plate from incubator and place in BSC.
18. Swirl or invert the tube of hepatocytes to ensure the cell suspension is homogeneously mixed, then pour the cell suspension into a sterile reagent reservoir until half full.
19. Aspirate medium from wells containing feeder cells, taking care not to disturb the feeder cell layer. For the 24-well format, proceed to Step 20. For the 96-well format, proceed to Step 21.
20. Using a multi-channel pipette, transfer 500 µL of cell suspension (300,000 cells) from the reservoir to each well of the 24-well culture plate. Gently shake the reservoir in a N-S, E-W orientation to ensure the cell suspension is homogeneous prior to each refill of the pipette. Proceed to Step 22.
21. Using a multi-channel pipette, transfer 100 µL of cell suspension (30,000 cells) from the reservoir to each well of the 96-well culture plate. Gently shake the reservoir in a N-S, E-W orientation to ensure the cell suspension is homogeneous prior to each refill of the pipette. Proceed to Step 22.
22. Remove culture plate from the BSC and return to the humidified incubator. For the 24-well format, proceed to Steps 23 and 24. For the 96-well format, do NOT shake the culture plate and proceed to Step 25.
23. Gently shake the culture plate in a figure 8 motion 5 times followed by a N-S, E-W orientation 5 times for even distribution of the hepatocytes. The shaking should be gentle, yet vigorous enough to evenly distribute the cells throughout the well.

24. Repeat the N-S, E-W motion every 15 minutes for the first 60 minutes of culture.

25. Place sufficient volume of supplemented TCCM in 37°C water bath to warm for 20-30 minutes prior to medium change. Protect from bright light.

26. At 2-4 hours post-plating, remove culture plate from incubator and inspect hepatocyte attachment. Place plate in BSC.

27. For the 24-well format, gently shake the plate in a N-S, E-W orientation to displace dead cells.

28. Tilt the plate and gently aspirate medium from the side of each well without touching the cell layer. Avoid prolonged or excessive aspiration of wells which may cause dehydration of the cells.

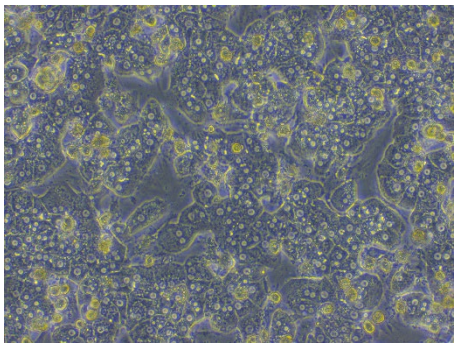
29. Replace medium in each well with 500 µL (24-well) or 100 µL (96-well) of warm supplemented TCCM, depositing the medium along the wall of the well, and return plate to incubator.

V. Maintenance of Rat Hepatocytes on Feeder Cells

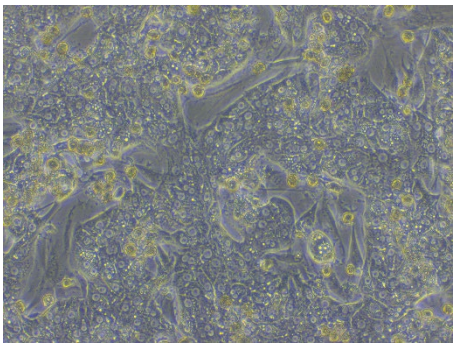
1. Visually inspect the culture daily.

2. Replace medium daily with 500 µL (24-well) or 100 µL (96-well) of warm supplemented TCCM per well for the remainder of the culture period. **NOTE:** Prepared supplemented TCCM can be stored at 4°C and used for 7 days. Fresh supplemented TCCM must be made weekly.

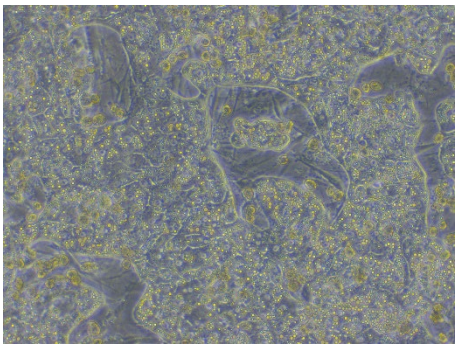
Representative Morphology During Week 1



Day 1: Hepatocytes have attached, and display expected cuboidal shape and multinucleation.



Day 4: Hepatocytes are beginning to aggregate together to form colonies.



Day 7: Hepatocytes have reached steady state and have formed well-defined colonies.

II. Week 2 Medium Preparation

1. Transfer Supplements D and E from -20°C to 4°C storage to thaw overnight. **OPTIONAL:** Thaw at room temperature or 37°C prior to use.

2. Prior to use, wipe reagent bottles with 70% (v/v) alcohol and place in BSC.

3. For the 24-well plate format, prepare TCCM by aliquoting 100 mL TCCM and adding 14 mL Supplement D, 1 mL Supplement E, and Dex to achieve a final concentration of 10 nM, or for the 96-well plate format by aliquoting 150 mL of TCCM and adding 22 mL Supplement D, 1.5 mL Supplement E, and Dex to achieve a final concentration of 10 nM. **NOTE:** If desired, an antibiotic can be added to TCCM. Penicillin/Streptomycin, at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.

4. Prior to use, warm only the amount of medium needed in 37°C water bath for 20-30 minutes.

Appendix I. Required Equipment and Consumables:

Large Equipment:

- Biological safety cabinet (BSC)
- Cryogenic storage freezer capable of temperatures ≤-150°C
- Portable liquid nitrogen (LN_2) dewar or other suitable container to transport frozen vials
- 37°C/5% CO_2 Incubator (humidified)
- 37°C Water bath
- Centrifuge capable of achieving up to 400 g (50 mL conical tube adaptors)
- -20°C freezer
- 4°C refrigerator
- Vacuum aspiration system with sterile aspiration tips (optional)

Small Equipment:

- Appropriate personal protective equipment (PPE)
- Laboratory ice tray capable of containing a small amount of liquid nitrogen and a cryovial box
- Hemocytometer or other cell-counting device
- Forceps or tongs
- Cryovial storage box
- Timer
- Pipettes (20, 200 and 1000 µL) with sterile tips
- Serological pipettor and sterile pipettes (1, 2, 5, 10, 25 mL)
- 1000 µL multichannel electronic pipette with sterile tips (optional)
- BioCoat™ Collagen I-coated 24-well culture plates (Corning cat. # 354408)
- Collagen I-coated 96-well plates (Fisher Scientific cat. #A1142803 or Corning cat. #354407)
- Sterile disposable or washable media bottles (100-250 mL)

Consumables:

- 70% (v/v) alcohol (ethanol or isopropyl alcohol)
- 70% alcohol wipes (or lab wipes soaked in 70% (v/v) alcohol)
- Laboratory wipes
- Ice
- Liquid nitrogen (LN_2)
- Sterile 15 mL and 50 mL conical centrifuge tubes
- Sterile 50 mL or 100 mL reagent reservoirs (optional)
- Sprague-Dawley or Wistar rat hepatocytes (recommendation - Thermo Fisher Scientific)
- Dexamethasone (10 mM stock; Fisher Scientific cat. #A13449)
- LifeNet Health Feeder Cell Thawing Medium (FCTM)
- LifeNet Health TruVivo Plating Medium (TCPM)
- LifeNet Health TruVivo Supplement B (TCSB)
- LifeNet Health TruVivo Culture Medium (TCCM)
- LifeNet Health TruVivo Supplement D (TCSD)
- LifeNet Health TruVivo Supplement E (TCSE)
- Antibiotic (optional; product suggestion - Penicillin/Streptomycin)
- Trypan blue solution or AO/PI (acridine orange/propidium iodide) stain

Appendix II. Quick Reference Guide TruVivo® Flex Kit for Rat Hepatocytes

I. Prepare Medium

1. Transfer FCTM and Supplements B, D, and E from -20°C to 4°C storage to thaw overnight. **OPTIONAL:** Thaw at room temperature or 37°C prior to use.
2. Prior to use, wipe reagent bottles with 70% (v/v) alcohol and place medium in biological safety cabinet (BSC).
3. Prepare TCPM by adding 4.5 mL Supplement B and 11 mL Supplement D to the bottle of TCPM. **NOTE:** If desired, an antibiotic can be added to the TCPM. Penicillin/Streptomycin, at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.
4. Prepare TCCM by aliquoting 100 mL of TCCM and adding 14 mL Supplement D, 1 mL Supplement E, and Dexamethasone to achieve a final concentration of 10 nM (24-well plate format). **NOTE:** If desired, an antibiotic can be added to the TCCM. Penicillin/Streptomycin, at a final concentration of 50 units/mL and 50 µg/mL, respectively, is recommended.
5. Prior to use, warm medium in 37°C water bath for 20-30 minutes.
6. Following addition of supplements to basal mediums, re-freeze Supplements D and E in a -20°C freezer until week 2 of culture.

II. Thaw and Plate Human Feeder Cells

1. Thaw feeder cells in 37°C water bath and transfer to FCTM.
2. Centrifuge at 400 x g for 4 minutes at room temperature.
3. Resuspend and count feeder cells.
4. Dilute cells to 100,000 cells/mL.
5. Plate feeder cells onto collagen I-coated plates - 500 µL/well (24-well) or 100 µL/well (96-well).
6. Place in humidified incubator and gently shake cells in N-S, E-W orientation (24-well). Do NOT shake 96-well format.
7. Incubate in humidified incubator for 60 minutes.
8. Visually inspect feeder cell attachment 60 minutes post-plating.

III. Thaw and Plate Rat Hepatocytes

1. Thaw rat hepatocytes in 37°C water bath and transfer to manufacturer recommended thawing medium.
2. Centrifuge following manufacturer recommended conditions.
3. Resuspend and count hepatocytes.
4. Dilute cells to 600,000 cells/mL (24-well) or 300,000 cells/mL (96-well).
5. Plate hepatocytes onto feeder cells - 500 µL/well (24-well) or 100 µL/well (96-well).
6. Place plate in a humidified incubator and gently shake in a figure 8 motion 5 times followed by N-S, E-W orientation 5 times (24-well). Do NOT shake 96-well format.
7. Repeat gentle shaking in incubator every 15 minutes for the first 60 minutes (24-well only).

IV. Medium Change

1. At 2-4 hours post-plating, gently shake plate and replace medium in each well with 500 µL (24-well) or 100 µL (96-well) TCCM.
2. Return plate to humidified incubator post-medium change.
3. Repeat medium changes for the duration of culture period.

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