BIOIVT

ELEVATING SCIENCE™

AccuLIVER™

CULTURE KIT

Instruction manual to culture TRANSPORTER CERTIFIED hepatocytes

BIOIVT
2810 Meridian Parkway, Suite 100, Durham, NC 27713 USA • Tel: 1-919-313-6500 • www.bioivt.com •

Version 1.1
August 2019

Table of Contents

About the ACCULIVER Culture Kit

The ACCULIVER™ Culture Kit contains media to culture TRANSPORTER CERTIFIED™ hepatocytes.

Technical Assistance

Contact BioIVT Transporter Solutions with questions about the ACCULIVER B-CLEAR® Kit, QUALGRO™ media, and this instruction manual.

Telephone: 919-313-6500 (Between 09:00 – 17:00 ET)

Contact Us: https://www.bioivt.com/about/contact-us/

Kit Contents

All materials in the kit must be stored at 4°C, except for the plates which may be stored at room temperature.

The ACCULIVER Culture Kit contains the following items:

- Collagen Coated 24-well plate (5)
- QUALGRO™ Seeding Medium (200mL)
- QUALGRO™ Overlay Medium (100mL)
- QUALGRO™ Culture Medium (250mL)

Other Required Reagents, Materials and Equipment

TRANSPORTER CERTIFIED Human Hepatocytes

TRANPSORTER CERTIFIED human hepatocytes are available from BioIVT. The quantity of cells required depends on the study design. Approximately 12 million cells are required per plate, assuming a seeding density of 1.0 million cells /mL.

Equipment and Materials

- Trypan Blue (Sigma, T8154)
- Matrigel™ (Corning, 354234)
- Biosafety Cabinet (BSL2)
- Humidified tissue culture incubator (37°C, 5% CO_2)
- Water bath (37°C)
- Slide Warmer (surface temperature set to 37°C)
- *Recommended:* 6-Channel EXP Impact2 Electronic Pipettor, 1250µL capacity (Matrix catalog # 2624), or equivalent**,** and compatible tips
- *Recommended:* 6-channel aspiration manifold (V.P. Scientific catalog # VP182C)
- *Recommended:* 6-channel partitioned reservoirs (FisherSci AWLS-S30030)

Cell Culture Study Protocol

Day 0: Thaw and plate hepatocytes

Thawing

1. Remove vial(s) of cryopreserved hepatocytes from liquid nitrogen storage
2. Immediately suspend vial(s) up to the cap in a water bath set for 37°C
3. Incubate vials in water bath for 1.5 – 2 minutes until the vials are ~90% thawed. There should still be a small, visibly frozen portion remaining in the vial(s).
4. Immediately remove vial(s) from the water bath, wipe down with ethanol and transfer to the tissue culture hood.
5. Transfer the contents of the thawed vials (up to ~ $40x10^6$ cells total or 4 vials) into 45mL of pre-warmed (37°C) Thawing Medium in a 50mL conical tube.
6. Rinse each vial 1 time by adding 1 mL of Thawing Medium from the conical tube to each vial and decanting the volume back into the 50mL conical tube.
7. Gently invert 50mL conical tube 3-5 times to mix.
8. Centrifuge at 100 x g for 8 minutes to pellet the cells.
9. Aspirate Thawing Medium supernatant.
10. Determine live cell yield and viability (Appendix 1).

See Appendix 2 for example pictures of hepatocytes in culture that have formed a proper matrix.

Plating

11. Dilute cell suspension with warm (37°C) Seeding Medium to the final concentration specified on the specific lot's TRANSPORTER CERTIFIED Data Sheet or Certificate of Analysis. (Plating density can vary from lot to lot and is specified on the data sheet.)
12. Gently agitate cell suspension to ensure uniform suspension of the hepatocytes.
13. Transfer 0.5mL of hepatocyte suspension to each well of a 24-well BD BioCoat plate.
14. After the suspension has been added to all the plates, place plates in a 37°C incubator and vigorously shake each plate in a north-south-east-west pattern ~10 times to evenly distribute the cells within each well.
15. Periodically agitate plates and if desired, examine plates under microscope to assess proper adherence. Proper adherence of cryopreserved hepatocytes takes approximately 2 – 4 hours from time of seeding.

Day 1: Overlay Hepatocytes

Hepatocytes must be overlaid 18 to 24 hours after seeding, using the ***cold*** (4°C) QUALGRO Overlay Medium, included in the kit. The following steps describe the overlay procedure:

1. Dilute Matrigel in cold (4°C) Overlay Medium to a final concentration of 0.25 mg/mL.
2. Remove the plates from the incubator for overlay.
3. Agitate plates to dislodge dead and/or poorly attached cells from the monolayer.
4. Aspirate the medium containing the dead/dislodged cells.
5. Add 0.5mL of Overlay Medium (containing Matrigel) to each well of the plates.

6. Nonspecific Binding Plates can be created by adding Overlay Medium (containing Matrigel) to previously empty wells in 24-well BD BioCoat plates as needed.

Days 2-4: Cell Culture Maintenance

Feed

Media needs to be exchanged every 24 hours.

Include test article depending on the study type and experimental design.

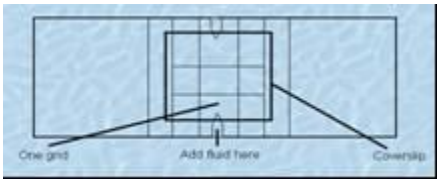
Image Cells (optional)

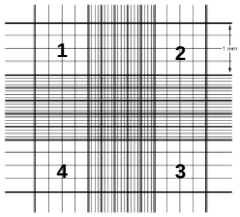
BioIVT recommends photographing the cells on every day of the study. Treatment groups can then be compared for signs of overt toxicity. Overt toxicity can impact data quality.

The morphology of the hepatocyte cultures should be compared to solvent controls for any morphological alterations (e.g., changes in cell shape, cytoplasmic alterations, accumulation of vacuoles suggestive of dilated organelles and lipid droplets) indicative of cytotoxicity (Tyson, 1987) (Guillouzo, 1997).

Appendix 1: Determining Live Cell Yield and Viability

1. Remove 50µL of cell suspension and place into a clean microcentrifuge tube.
2. Add 400µL of the Seeding Medium
3. Add 50µL of Trypan Blue (Sigma, T8154) and mix by gently inverting tube several times (dilution factor = 10).
4. Place a clean cover slip over the chamber of the hemocytometer.
5. Fill one or both sides of the chambers with 10µL of cell suspension containing Trypan Blue. View under a microscope using 10X magnification. (Example of four squares being counted)
6. Count the number of viable cells (seen as bright cells) and non-viable cells (stained blue).
7. Calculate % viability:





$$[Percentage\ of\ Viable\ Cells] = \frac{[Total\ Number\ of\ Viable\ Cells]}{[Total\ Number\ of\ Cells]} \times 100$$

8. Calculate the number of cells/mL:

$$[Number\ of\ Cells\ /mL] = \frac{[Total\ Number\ of\ Viable\ Cells]x[dilution\ factor]x10^4}{Number\ of\ Squares\ Counted}$$

9. Calculate total number of cells:

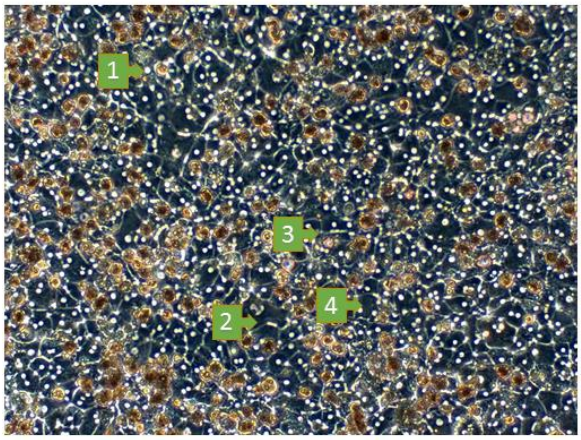
$$[Total\ Number\ of\ Cells] = [Cells\ per\ mL]x[Total\ Liquid\ Volume]$$

Appendix 2: Comparison of High Versus Low Quality Cultures of Hepatocytes

By Day 2 of culture, hepatocytes should begin to polarize and form bile pockets. Cells should be > 90% confluent and 100% confluent is ideal. Below is an example comparing good vs bad cultures of hepatocytes. Poor quality cultures should not be used in the study and should be discarded.

Hepatocyte Cultures

High Quality Culture
1. Clear, distinct nuclei
2. Clear cytoplasm: no swollen organelles; no intracellular debris.
3. Confluent monolayer: indicates tight cell-to-cell junctions, formation of bile canaliculi
4. Normal cell shape



Low Quality Culture
1. Indistinct nuclei
2. Swollen organelles and intracellular debris
3. Gaps in the monolayer; limited cell-to-cell interaction
4. Elongated, fibroblast-like cells