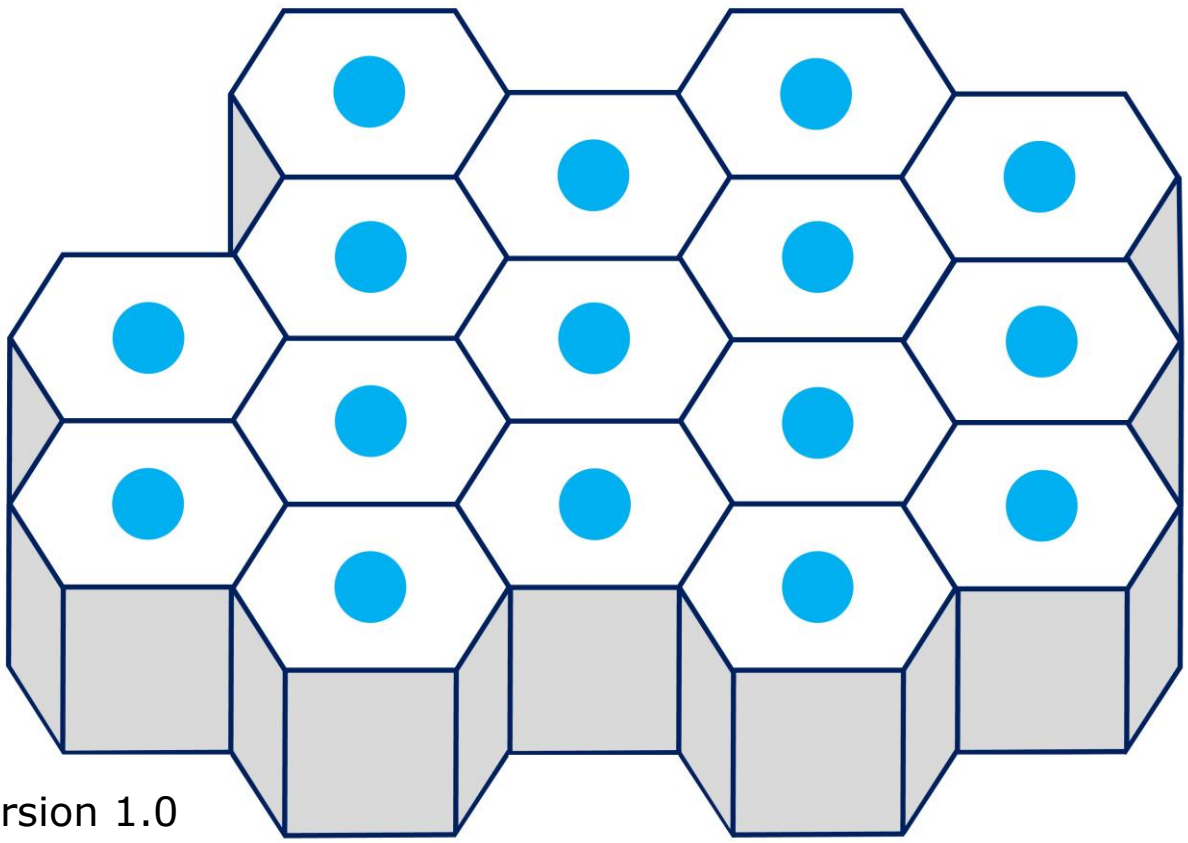
User Guide

Primary Human Hepatocytes

Preparation of Primary Human Hepatocytes

Cat# HH1, HH2 & HH3



Protocol Version 1.0

Contents

Preparation of Primary Human Hepatocytes

Materials and Equipment Required

- Heated water bath
- Serological pipettes
- Micropipettes
- Centrifuge with rotor for 15 or 50 mL tubes
- 37°C incubator with 5% CO2
- Ice box
- Nageotte cassettes

Reagents Supplied

- Hepatocyte thawing medium
- Additives for hepatocyte thawing medium
- Hepatocyte culture medium
- Additives for hepatocyte culture medium
- Trypan blue solution

Storage

It is critical to store the cells in temperatures lower than -150°C. DO NOT store cells at -80°C or on dry ice.

Use personal protective equipment: lab coat, gloves and goggles when working with cell cultures.

Thawing Hepatocytes

1. Prepare the hepatocyte thawing medium by adding the additives for thawing medium to 20 mL of hepatocyte thawing medium. Prewarm hepatocyte thawing medium to 37°C before use.

2. Prepare hepatocyte culture medium by adding additives for cultivation medium to 10 mL of hepatocyte cultivation medium. Keep cultivation medium at 4°C.

3. Remove the required number of cryovials containing the frozen hepatocytes from the liquid nitrogen tank.

4. Quickly place the cryovials in a 37°C water bath (DO NOT use an incubator). Slightly open the vial to remove any excessive liquid nitrogen.

5. While holding the tip of the cryovial containing the hepatocytes, gently agitate by hand, not allowing water to penetrate through the cap, to ensure uniform defrosting. DO NOT submerge the cryovial completely in water.

6. Thaw the cryovial of cells in a water bath until only a small ice pellet remains (this usually takes around 80-115 seconds).

7. Immediately remove the cryovial from the water bath. Wipe the outside of the cryovial bottle with an alcohol wipe and place in a sterile laminar flow cabinet.

8. Transfer the contents of the cryovial (including the cells) to the pre-warmed hepatocyte thawing medium.

9. Carefully rinse the cryovial with 1 mL of hepatocyte thawing medium and transfer the remaining cell suspension to a vial.

10. Gently mix the cell suspension in the vial by pipetting (DO NOT vortex).

11. Pellet the cells by centrifuge at 200 x *g* for 6 minutes at room temperature

12. Carefully aspirate the supernatant without disturbing the cell pellet.

13. Resuspend the cell pellet in 3-4 mL of cold cultivation medium.

14. Measure the exact volume of the cell suspension with a 5 mL graduated pipette. The cell suspension should be stored on ice or at 4°C during all the following procedures.

Evaluating Cell Count and Viability

Hepatocytes can be counted using your routine cell counting method. This protocol outlines a precise method for quantifying hepatocytes using Nageotte chambers, a well-established and dependable technique frequently used in our laboratory settings.

1. Prepare a Nageotte counting chamber with coverslip.

Note: carefully clean the counting chamber (the mirror-like polished surface) with paper. Then clean and place the coverslip over the counting surface prior to putting on the cell suspension.

2. Homogenize the cell suspension by manually swirling the cell bottle gently (DO NOT vortex).

3. Dilute 50 µL of the cell suspension into 950 µL of trypan blue solution, keeping the rest of the cell suspension on ice.

Note: the appropriate dilution of the cell suspension will allow the cells to be uniformly distributed.

4. Homogenize the mixture of cell suspension with Trypan blue by gently inverting the tube to ensure a uniform cell suspension (DO NOT vortex).

5. Using a pipette add 100 µL of cell suspension between the mirror-like polished surface and coverslip.

Note: enough liquid should be introduced so that the mirrored surface is just covered with mixed cell suspension.

6. Proceed to cell observation under the microscope to determine total cell yield, percent viability, and cell integrity.

Note: living cells exclude the dye; dead cells take up the dye and appear blue. If the total number of cells is very different from one row to another, count one or two more rows.

7. Count living and dead cells on at least two columns of the Nageotte chamber with an average of 100-300 cells per row (3-10 million cells/mL).

8. Determine the average number of viable cells and dead cells per row.

a. Determine percentage of cell viability:

$$Cell\ viability\ (\%) = \frac{Number\ of\ viable\ cells}{Number\ of\ viable\ cells + Number\ of\ dead\ cells} \times 100$$

b. Calculate the cell concentration (Million cells/mL) with a Nageotte chamber:

$$Cell\ concentration\ (m/mL) = Number\ of\ viable\ cells\ per\ row \times 40\ (dilution\ factor\ in\ Trypan\ blue) \times 800\ (Nageotte\ factor)$$

Plating Human Hepatocytes (only for HH1)

- Seed cells on a collagen-coated 24-well plate in concentrations of 600 and 800 thousand per well.
- For use in a 96-well plate seed 70,000-90,000 cells/well depending on the experimental requirements.

Seeding Human Hepatocytes for Spheroid Formation (only for HH3)

- Distribute the cells on an Ultra-Low Attachment 384-well plate at 10,000 viable cells per well.
- Centrifuge the well-plate at 50 x g for 3 minutes.
- Do not disturb the cells for 7-10 days until proper spheroids have formed. Spheroids should have a clearly visible membrane on the surface. Do not start your experiments unless all wells have homogeneously formed such membrane.

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