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ADAPTATION OF HUMAN HEPATOCELLULAR CARCINOMA (HUH7) CELLS MAINTAINED IN HIGH-GLUCOSE DULBECCO'S MODIFIED EAGLE MEDIUM (DMEM) TO ROSWELL PARK MEMORIAL INSTITUTE (RPMI)-1640 MEDIUM

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We use this protocol and it's working

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Abstract

This is a standard operating procedure (SOP) for thawing, cultivating, adapting, and freezing the Huh7 cells by the antiviral laboratory in the Department of Medical Microbiology and the Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya, Malaysia. Huh7 cells are actively used in antiviral and cancer research as they offer a suitable environment for the replication of various viruses and serve as a reliable model for hepatocellular carcinoma studies. Like many other cell lines, Huh7 cells can be grown in different types of media depending on the specific requirements of a particular experiment or study. There are instances when one might need to adapt Huh7 cells grown in high-glucose DMEM to RPMI-1640 such as experimental requirements, viral replication, media composition differences, cell physiology and behavior, comparative studies, and standardization across laboratories. Adapting cells from one medium to another is not always straightforward. Cells might initially show reduced growth rates and may need time to acclimate to the new conditions. Proper adaptation procedures should be followed to ensure that the cells remain viable and maintain their essential characteristics during the transition. Regular training and adherence to this protocol will ensure that the Huh7 cells retain their significance as a valuable tool in antiviral and cancer research.

Guidelines

1. Always use aseptic techniques to prevent contamination. Work in a biosafety cabinet (BSC) whenever possible, regularly disinfect the work area, and use sterile equipment and reagents.
2. Regularly check the cells under a microscope for morphology and signs of contamination. Use assays like the trypan blue exclusion test to assess cell viability.
3. Always ensure that the culture conditions (temperature, CO2 concentration, humidity) in the incubator are optimal for the cells growing.
4. Change the media regularly to provide cells with fresh nutrients and remove waste products.
5. Avoid letting the cells become over-confluent. Regularly passage the cells to maintain them in their logarithmic growth phase, which ensures they remain responsive and viable.
6. Maintain a detailed lab notebook with the date, passage number, media changes, observations, and any treatments or changes made to the culture.
7. Always keep backup vials of cells in liquid nitrogen. This ensures you have a backup in case of contamination or other issues with the active culture.
8. Handle cells according to the appropriate biosafety level (BSL) guidelines. Ensure that any potentially biohazardous material is properly decontaminated before disposal.
9. Only trained individuals should handle and work with cell cultures. Regular refresher courses or training can be beneficial.
10. Work towards standardizing protocols as much as possible. This ensures that experiments can be replicated, either within the same laboratory or across different labs.
11. Properly dispose of cell culture waste. This often means autoclaving waste before disposal, especially if the cells were used for pathogenic studies.

Materials

Cells

Well-differentiated human hepatocellular carcinoma cell line, Huh7 cells were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank of the National Institute of Biomedical Innovation, Health, and Nutrition (NIBIOHN). Upon receiving in the form of frozen cells, the cells are cultivated in high-glucose DMEM supplemented with 20% fetal bovine serum (FBS) at 2.5 × 104 cells/cm2 in a 25cm2 tissue culture flask. The cells are incubated at 37°C with ±5% CO2 and 95% humidity and checked daily.

Initial growth medium

High-glucose DMEM (Gibco 11965092)
10% FBS (Gibco 10100147)
100 IU/ml Penicillin-streptomycin (P/S) (Sigma-Aldrich P4333)
Non-essential amino acid (NEAA) (Gibco 11140050)
2mM L-glutamine (Gibco 25030081)

Final growth medium

RPMI-1640 (ATCC 30-2001™)
10% FBS
100 IU/ml P/S
NEAA
2mM L-glutamine

Freezing medium

70% Initial or final growth medium without FBS
10% Dimethyl sulfoxide (DMSO) (Sigma-Aldrich D2650)
20% FBS

Passaging reagent

1x Phosphate buffer saline (PBS)
1.2% Trypsin-versene (T/V)
Trypan blue

Plasticware

5ml and 10ml serological pipettes
1ml pipette tip
1ml, 15ml and 50ml centrifuge tubes
25cm2
tissue culture flask
Hemocytometer
500ml beaker
2ml cryovials

Equipment
Class II type A2 BSC
Incubator (cell house)
Inverted microscope
Water bath
Liquid nitrogen
Freezing container
Pipette aid/gun
-80°C freezer
Water bath

Troubleshooting

Safety warnings

1. Ensure that proper PPE is worn at all times (lab coat or lab gown, safety glasses if handling eye hazards, gloves, proper enclosed footwear, tie long hair)
2. Ultraviolet lights in the biosafety cabinet pose a burn hazard to the skin and a blindness hazard to the eyes. UV light can cause DNA damage leading to cancer. Ensure UV lights are off before starting work.
3. Ethanol and other disinfectants can be toxic and/or flammable. Know the risks associated with each reagent before you start work by reading the SDS.
4. Tissue culture may pose a biohazard risk depending on what it is used for, e.g. when used to propagate viruses, this can be a serious human health risk. Know the risks associated with the biohazards before starting work.
5. Tissue culture work is typically done inside a biosafety cabinet or other protective cabinet. Ensure you also read the correct risk assessment and SOP for the type of cabinet you are using.
6. The CO2 gas used in the tissue culture incubator can pose an asphyxiation or toxicity risk if inhaled. Ensure you have also read and understood the risk assessment and SOP for working with compressed gases. If you have any evidence that a CO2 leak is occurring or imminent, leave the room immediately and consult your supervisor and/or the room custodian.
7. Know the protocols for disposing of cell culture waste and any other hazardous materials that might be produced.
8. Be prepared for potential setbacks, like contamination or equipment malfunction. Have a plan to address these issues if they arise.

Before start

1. Clearly understand the purpose of culturing the cells. Is it for a specific assay, viral replication, gene expression, or another reason? (Your goals dictate how you'll handle and treat the cells).
2. Familiarize yourself with the Huh7 cell line's characteristics. This includes its morphology, growth rate, common contamination issues, and specific requirements for optimal growth.
3. Understand the differences between DMEM and RPMI 1640. Knowing the key components and their concentrations can help anticipate how the cells might respond during adaptation.
4. Some cell lines may require additional supplements (e.g., serum, growth factors, or antibiotics) in their culture media. Know what the Huh7 cell line needs and ensure its availability.
5. Operate all equipment correctly, from the CO2 incubator to the biosafety cabinet, and microscope.
6. Read the entire protocol from start to finish before beginning. This can prevent mistakes and ensure all necessary materials and reagents are available. Also, ensure all reagents are within their expiration dates.

Thawing Huh7 cells

1 Remove the cryovial of Huh7 cells from liquid nitrogen storage and quickly place it into a 37°C water bath. Gently shake the vial to hasten thawing.

2 Once thawed (within 1-2 minutes), clean the outside of the cryovial with 70% ethanol.

3 Transfer the thawed cells into a flask containing 4 ml of pre-warmed initial growth medium with 20% FBS and swirl gently.

4 Keep the flask at 37°C with ±5% CO2 and 95% humidity and checked daily.

Cultivating Huh7 cells

5 Monitor cell growth daily using an inverted microscope. Cells should be sub-cultured upon reaching ~80% confluency.

6 To sub-culture, aspirate old media, wash cells with 2ml PBS, and add 0.5ml T/V solution.

7 While trypsinizing, keep the flask at 37°C with ±5% CO2 and 95% humidity for 3 minutes.

8 After that, remove the flask from the incubator and gently tap the edges of the flask in the BSC.

9 Add 4.5ml pre-warmed initial growth medium to stop the reaction of T/V.

10 Perform the trypan blue exclusion test to assess cell viability.

11 Split the number of cells at a 1:1 ratio (2.5ml in each flask) and add 2.5ml of growth medium.

12 Keep the flask at 37°C with ±5% CO2 and 95% humidity and checked daily.

Adapting Huh7 cells

13 For the first five passages, maintain cells in 100% initial growth medium.

14 From passages 6 to 10, use a mixed volume of 80% initial growth medium and 20% final growth medium.

15 From passages 11 to 15, use a mixed volume of 60% initial growth medium and 40% final growth medium.

16 From passages 16 to 20, use a mixed volume of 40% initial growth medium and 60% final growth medium.

17 From passages 21 to 25, use a mixed volume of 20% initial growth medium and 80% final growth medium.

18 From passage 26 onwards, maintain cells in 100% final growth medium.

Freezing Huh7 cells

19 Once cells reach ~80% confluency, trypsinize and pellet as detailed in the "Cultivating" section.

20 Resuspend the cell pellet in the freezing medium without the DMSO.

21 Aliquot the cell suspension into cryovials. Add DMSO drop-by-drop into the cryovials until the desired volume is reached (the addition of DMSO creates heat in the freezing media and adding it drop-by-drop will reduce the chance of cell unviability).

22 Place cryovials in a freezing container to ensure a controlled freezing rate.

23 Transfer the freezing container to a -80°C freezer overnight.

24 The next day, move the cryovials to liquid nitrogen storage for long-term preservation.