HPB-16 E6/E7 protein expression

Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ atmosphere is recommended if using the medium described on this product.

It is important to note that some vials leak when submersed in liquid nitrogen vapor phase and not at 70°C. Storage at 70°C will result in loss of viability.

To make the complete growth medium, add the following components to the base medium:
- 0.1 mg/mL G-418
- additional 2 mM Glutamine
- heat-inactivated fetal bovine serum (FBS) to a final concentration of 10%

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor, not at 70°C. Storage at 70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a new culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer, twice, with 5 mL Ca++/Mg++ free Dulbecco's phosphate-buffered saline (DPBS) or 0.05% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contain trypsin inhibitor.
3. Add 5 mL of 0.05% Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 5 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new vessels. An inoculum of 1 X 10^4 to 2.0 X 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37°C.

Subcultivation ratio: A subcultivation ratio of 1:2 to 1:3 is recommended.

Medium renewal: Every 2 to 3 days

Comments

PHM1-41 cells retain many morphological and phenotypic responses in common with cultured primary myometrial cells. They are useful for long-term or coordinated studies, as their behavior is quite reproducible and the alternative, obtaining material from patients, is labor intensive and variable. These cells have been used to study crosstalk signaling between contractant and relaxant pathways, oxytocin receptor signaling pathways, and signal regulated control of calcium dynamics in the cytosol and endoplasmic reticulum compartments. Furthermore the can be used for studies on ion channel activity, expression, and gene regulation.

PHM1-41 does not grow in soft agar.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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