Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature If the cells are still attached,

If the cells are not attached,

It is important to note that some vials leak when submersed in liquid nitrogen

Biosafety Level

Check all containers for leakage or breakage.

Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at Incubate the culture at 37°C in a suitable incubator. A 5% CO

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: F4 G6PD Knockout (ATCC® CRL-3309™)

Handling Procedure for Frozen Cells

To ensure 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 phase and 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 150 x g for 8 to 12 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 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). 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 sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. If the cells are still attached, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.
Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with DPBS (ATCC catalog # 30-2200) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL 0.25% (w/v) Trypsin-0.53 mM 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 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
   Cultures can be established between 2x 10⁶ and 5 x 10⁶ viable cells/cm².
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 2 X 10⁶ and 2 X 10⁵ cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week

Cryopreservation Medium

Complete media + 10% DMSO

Comments

This cell line was created from the mouse liver cell line BNL CL.2 (ATCC® TIB-73™) by using CRISPR/Cas9 genome editing to knock out glucose 6-phosphate dehydrogenase (G6PD). G6PD is an important enzyme participating in the pentose phosphate pathway to provide reducing power NADPH. The complete knock out of G6PD within this cell lines have been determined by DNA sequencing, enzyme activity assays, and western blotting. BNL CL.2 High passage (ATCC® CRL-3308™) is to be used as a control for F4 G6PD Knockout cell line.

References

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

Biosafety Level: 1

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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Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The complete medium for this cell line comprises DMEM with 10% FBS that has been heat inactivated at 56°C for 30 minutes. To make the complete medium add 500 mL DMEM (ATCC® 30-2002™) to 56 mL heat inactivated FBS (ATCC® 30-2020™). Note: Serum after heat inactivation is aliquoted in 50 mL aliquots at -20°C. Thaw at 37°C before adding to the media.

Citation of Strain

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