

NCI-H2009 | 305283

Cell Line Information

Viruses Epstein-Barr virus (EBV)

Mutational profile B2M p.Met1Val (c.1A>G) B2M p.Gln28Ter (c.82C>T) KRAS p.Gly12Ala (c.35G>C) TP53 p.Arg273Leu (c.818G>T)

Media

Culture Medium HITES

- 0.005 mg/ml insulin
- 0.01 mg/ml transferrin
- 30 ng/ml selenium (selenium supplement)
- 10 ng/ml hydrocortisone (hydrocortisone supplement)
- 10 ng/ml progesterone (progesterone supplement)
- 2 mg/ml L-ascorbic acid (ascorbic acid supplement 4.5 mg/ml)
- 5 mg/ml sodium selenite (selenium supplement)

Supplements 5% FBS 0.005 mg/ml insulin 0.01 mg/ml transferrin 30 ng/ml selenium 10 ng/ml hydrocortisone 10 ng/ml progesterone

Dissociation Reagent Trypsin

Subculturing Cells are cultured in HITES medium supplemented with 5% FBS. For subculturing, cells are trypsinized and resuspended in HITES medium.

Split ratio 1:3 or 1:6

Fluid renewal 2-3 times per week

Freeze medium HITES medium supplemented with 5% FBS + 10% DMSO

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Thawing and Culturing Cells

1. Thaw the vial rapidly in a water bath at 37°C. Remove the vial and centrifuge at 300 × g for 3 minutes. Discard the supernatant and resuspend the cells in 10 ml of complete medium. Seed the cells into a T75 flask containing 50 ml of complete medium. Incubate the cells for 24 hours to allow them to attach to the flask.
2. After 24 hours, check the cells under a microscope. If the cells are not attached, discard the medium and repeat the seeding process.
3. Once the cells are attached, replace the medium with fresh complete medium. After 24 hours, the cells should be at 70% confluency.
4. When the cells reach 70% confluency, they can be passaged. Remove the medium and wash the cells with PBS. Add 1 ml of trypsin-EDTA solution and incubate for 5 minutes at 37°C. Add 9 ml of complete medium to stop the trypsin. Harvest the cells by centrifugation at 300 × g for 3 minutes. Resuspend the cells in 1 ml of complete medium and count them.
5. Seed the cells into a T75 flask containing 50 ml of complete medium. Incubate the cells for 24 hours to allow them to attach to the flask.
6. After 24 hours, check the cells under a microscope. If the cells are not attached, discard the medium and repeat the seeding process.
7. Once the cells are attached, replace the medium with fresh complete medium. After 24 hours, the cells should be at 70% confluency.
8. When the cells reach 70% confluency, they can be passaged. Remove the medium and wash the cells with PBS. Add 1 ml of trypsin-EDTA solution and incubate for 5 minutes at 37°C. Add 9 ml of complete medium to stop the trypsin. Harvest the cells by centrifugation at 300 × g for 3 minutes. Resuspend the cells in 1 ml of complete medium and count them.

Incubation Atmosphere 37 °C, 5% CO₂, humidified

Flask Coating None

Shipping Conditions Dry ice, -78 °C

Storage Conditions -150 °C to -196 °C

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Sterility Sterility testing: PCR