NRAS-mutant-A375 Isogenic-Luc2 (ATCC® CRL-1619IG-2-LUC2™)

**Description**

**Organism:** Homo sapiens, human  
**Tissue:** skin  
**Disease:** malignant melanoma  
**Age:** 54 years  
**Gender:** female  
**Morphology:** epithelial  
**Growth Properties:** adherent

**DNA Profile:**
- Amelogenin: X
- CSF1PO: 11,12
- D13S317: 11,14
- D16S539: 9
- D5S818: 12
- D7S820: 9
- TH01: 8
- TPOX: 8,10
- vWA: 16,17

**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: NRAS-mutant-A375 Isogenic-Luc2 (ATCC® CRL-1619IG-2-LUC2™)

**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 base medium for this cell line is Dulbecco's Modified Eagle's Medium (DMEM; ATCC 30-2002). To make the complete growth medium, add the following components to the base medium:
- Fetal bovine serum (FBS; ATCC 30-2020) to a final concentration of 10%
- Blasticidin to a final concentration of 8 µg/mL

**Handling Procedure for Frozen Cells**

1. Check all containers for leakage or breakage.
2. 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.
3. 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).
4. 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**

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.
2. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for...
If the cells are still attached, briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of components to the base medium:

- Fetal bovine serum (FBS; ATCC 30-2020) to a final concentration of 10%
- Blasticidin to a final concentration of 8 µg/mL

Citation of Strain

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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 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of 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 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 1 x 10⁴ and 4 x 10⁴ viable cells/cm².
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 8 x 10⁴ and 4 x 10⁴ cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:6 is recommended

Medium Renewal: 2 to 3 times per week

Cryopreservation Medium

Complete growth medium supplemented with 5% (v/v) DMSO

Comments

This luciferase expressing cell line was derived from parental line CRL-1619IG-2 by transduction with lentiviral vector encoding firefly luciferase gene (luc2) under control of EF-1 alpha promoter. The parental line CRL-1619IG-2 is a CRISPR gene edited melanoma drug resistant model containing both BRAF V600E and NRAS Q61K mutations. This cell line was established through single cell cloning, and the cells constitutively express high levels of enzymatically active luciferase protein, which can be detected via in vitro and in vivo bioluminescence assays. The cells should be maintained in Blasticidin (8 µg/mL) containing medium in routine cell culture. It is recommended to remove Blasticidin prior to and during the experiment procedure when the cells are injected into animals in vivo, or co-cultured with other cell types in vitro.

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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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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