### Handling Procedure for Frozen Cells

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep it on ice during thawing.
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.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 150 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see 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).
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

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...
If the cells are still attached, aseptically remove the entire contents of the flask and centrifuge at 37°C. 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 they are ready to be subcultured.

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 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.
6. Incubate cultures at 37°C.

**Interval:** Maintain cultures at a cell concentration between 8 X 10³ 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
- Every 4 to 5 days

### Cryopreservation Medium

Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

**Comments**

This is a malignant BRAFV600E/MEK1Q56P mutant isogenic line derived from the parental A375 (ATCC CRL-1619) cell line. The c.167A>C knock-in mutation encoding MEK1 p.Q56P protein expression was generated at ATCC by utilizing the CRISPR/Cas9 gene editing technology. This is a heterozygous mutation expressing the MEK1 wild-type and the c.167A>C mutant alleles.

**BRAF** is a proto-oncogene encoding B-RAFT, a serine/threonine kinase of the RAF family that acts downstream of RAS and upstream of MEK in the MAPK/ERK signaling pathway. Mutations in BRAF lead to excessive cellular proliferation, differentiation, and survival. BRAF V600E mutations are present in 50% of melanomas and although there are current BRAF inhibitors used as successful therapeutics, patients often become resistant to drugs several months following treatment. One mechanism of resistance to these inhibitors is caused by upstream secondary RAS acquired mutations. The MEK1 Q56P mutant isogenic line, ATCC CRL-1619IG-3 has been validated at the genomic, transcript, and protein bio-functional levels and exhibits significant resistance to the BRAF inhibitors Dabrafenib and Vemurafenib, as well as the MEK inhibitor trametinib when compared to its parental cell line. Furthermore, A-375 MEK1Q56P (ATCC CRL-1619IG-3™) cells display increased sensitivity to combination MEK/BRAF inhibitor treatments, making this line an ideal model system for the development of novel combination therapies targeting multiple points in the RAS-RAF-MEK-ERK-MAPK signaling pathway, as well as for the screening of new potential BRAF and MEK inhibitors.

### References

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