Product Sheet
IDH1 mutant-U-87 Isogenic Cell Line (ATCC® HTB-14IG™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

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 Eagle's Minimal Essential Medium (EMEM; ATCC 30-2003). To make the complete medium add Fetal Bovine Serum (FBS; ATCC 30-2020) to the base medium for a final concentration of 10%.

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: IDH1 mutant-U-87 Isogenic Cell Line (ATCC® HTB-14IG™)

Description
Organism: Homo sapiens, human
Tissue: brain
Disease: glioma
Gender: male
Morphology: epithelial
Growth Properties: adherent

DNA Profile:
Amelogenin: X
CSF1PO: 10,11
D13S317: 8,11
D16S539: 12
D5S818: 11,12
D7S820: 8,9
TH01: 9,3
TPOX: 8
vWA: 15,17

Batch-Specific Information
Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION
ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

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 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 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 5 to 7 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.

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
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 2 X 10^4 and 1 X 10^5 cell/cm².

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

Medium Renewal: 2 to 3 times per week

**Complete Growth Medium**

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

**Comments**

This is a glioma IDH1R132H mutant isogenic line derived from the parental U-87MG (ATCC® HTB-14) cell line. The c.395G>A knock-in mutation encoding IDH1R132H protein expression was generated at ATCC by utilizing the CRISPR/Cas9 gene editing technology. This is a heterozygous mutation expressing the c.395G>A mutant allele. The IDH1R132H mutation in HTB-14IG has been validated at the genomic, transcript, and protein biochemical levels.

The IDH1R132H mutation is among the most common seen in glioma and serves as an important diagnostic marker in various stages of disease in patients. This IDH1R132H mutant isogenic line HTB-14IG has been tested at ATCC for neomorphic functional activity displaying elevated levels of intra- and extracellular D-2HG above the parental U-87MG line. In addition, the HTB-14IG IDH1 mutant isogenic line displays histone hypermethylation compared to the parental line. This IDH1R132H isogenic cell model is a valuable in vitro cell-based tool for clinical diagnostics, elucidating mechanisms involved in cancer-associated differentiation, tumorigenesis and use in screening anti-cancer compounds for drug discovery and development. Refer to the parental line U-87MG (ATCC® HTB-14) for additional background information.

**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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**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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

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