Incubate the culture at 37°C in a suitable incubator. A 5% CO

Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by

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

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

The base medium for this cell line is:
  ● 50% ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001.
  ● 50% MEGM (Mammary Epithelial Growth Medium from Clonetics/Lonza (MEGM Bullet Kit; CC-3150) made of MEBM basal medium and SingleQuot additives (ATCC does not use gentamycin-amphotericin B).

Note: Do not filter complete medium. To make the final complete growth medium add the following components to the base medium:
  ● G-418 to a final concentration of 200ug/ml.
  ● fetal bovine serum to a final concentration of 3%.

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: UWB1.289+BRCA1 (ATCC® CRL-2946™)

Citation of Strain

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 submerged 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.

To insure 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, below -130°C, preferably in liquid nitrogen vapor, until ready for use.

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.

SAFETY PRECAUTION

Handling Procedure for Frozen Cells

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 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which 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 2.0 to 3.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to culture vessels. An inoculum of 5 x 10⁶ to 7 x 10⁶ viable cells/cm² is recommended.
7. Incubate cultures at 37°C. Subculture when cell concentration is between 4 x 10⁵ and 6 x 10⁵ cells/cm².

### Subcultivation ratio

A subcultivation ratio of 1:4 to 1:6 is recommended.

### Medium renewal

- Every 2 to 3 days

### Cryopreservation Medium

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

Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

### Comments

A pcDNA3 plasmid carrying wild-type BRCA1 was transfected into the parent line. Restoration of wild-type BRCA1 function in these cells partially restores DNA damage responses. ref

Restoration of wild-type BRCA1 function in these cells partially restores DNA damage responses.

### 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.

### ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.
Intended Use

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Complete Growth Medium

The base medium for this cell line is:
- 50% ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001.
- 50% MEGM (Mammary Epithelial Growth Medium from Clonetics/Lonza (MEGM Bullet Kit; CC-3150) made of MEBM basal medium and SingleQuot additives (ATCC does not use gentamycin-amphotericin B).

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