**Product Sheet**

10.014 pRSV-T (ATCC® CRL-11515™)

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**Description**

Organism: *Homo sapiens*, human  
Tissue: eye, cornea  
Cell Type: epithelial cells transfected with a plasmid containing the SV40 early region  
Morphology: epithelial  
Growth Properties: adherent

DNA Profile:
- **Amelogenin**: X,Y
- **CSF1PO**: 10
- **D13S317**: 12,13
- **D16S539**: 8,13
- **D5S818**: 12,13
- **D7S820**: 8,10
- **TH01**: 6,8
- **TPOX**: 8,10
- **vWA**: 15

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**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 Keratinocyte-Serum Free Medium (ThermoFisher cat # 17005-042) with 5 ng/ml human recombinant EGF, 0.05 mg/ml bovine pituitary extract. To make the complete medium add the following components to the base medium:
- 0.005 mg/ml human recombinant insulin (ThermoFisher cat # 12585-014) and 500 ng/ml hydrocortisone (Sigma cat # H0135).

**Release Information**

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: 10.014 pRSV-T (ATCC® CRL-11515™)

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**Batch-Specific Information**

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

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**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 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.

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**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.

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**Handling Procedure for Frozen Cells**

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 phase and not at -70°C. Storage at -70°C will result in loss of viability.

The flasks used must be precoated with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin.

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 15 mL centrifuge tube and dilute with the recommended complete culture medium.
4. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh medium at the dilution ratio recommended in the specific batch information on a pre-coated flask. 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 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.

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**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 precoated 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.

**Note:** The flasks used should be precoated with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin. **Subculture when 80% confluent.**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.05% Trypsin-0.53 mM EDTA (ThermoFisher cat# 25300-054).
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 with 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 fresh medium and aspirate cells by gently pipetting.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new coated culture vessels.
7. Place culture vessels in incubators at 37°C.

### Subculture Ratio:
A subculture ratio of 1:2 to 1:4 is recommended when 80% confluent

### Medium Renewal: Every 2 to 3 days.

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

### Cryopreservation Medium

The cells are frozen in complete growth medium described above supplemented with 7.5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

### Comments

On a personal communication with the ATCC, the depositor commented that these cells have a finite life span.

**Depositor Reference:**

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

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