# ASC52telo, hTERT immortalized adipose derived Mesenchymal stem cells

## SCRC-4000™

### Description

ASC52telo is an hTERT immortalized adipose derived Mesenchymal stem cell line exhibiting a fibroblast-like morphology that was isolated in 2006 from the adipose tissue of a White female. The cell line can be used in stem cell research.



**Organism:** *Homo sapiens*, human
**Cell Type:** mesenchymal stem cell
**Tissue:** Adipose tissue
**Gender:** Female
**Morphology:** fibroblast-like
**Growth properties:** Adherent

### Storage Conditions

**Product format:** Frozen
**Storage conditions:** Vapor phase of liquid nitrogen

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## Intended Use

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

## BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Cells contain SV40 sequences

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

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## Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% $CO_2$

## Handling Procedures

**Unpacking and 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.

**Complete medium:** The base medium for this cell line is Mesenchymal Stem Cell Basal Medium (ATCC PCS-500-030). To make the complete growth medium, add Mesenchymal Stem Cell Growth Kit (ATCC PCS-500-040) for Adipose and Umbilical-derived MSCs - Low Serum Components and G418 to the base medium as the following:

482 mL of basal medium (PCS-500-030)

10 mL of MSC supplement (2% FBS, 5 ng/mL rh FGF basic, 5 ng/mL rh FGF acidic, 5 ng/mL rh EGF)

6 mL of L-Alanyl-L-Glutamine (2.4 mM, final concentration)

2 mL of 50 mg/mL G418 (0.2 mg/mL, final concentration)

**Handling Procedure:**

**Recovery of Frozen Cells**

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and **NOT** at -70°C. Storage at -70°C will result in loss of viability.

**SAFETY PRECAUTION: Always use protective gloves and clothing and wear a full face mask when handling frozen vials.** Some vials leak when immersed 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.

1. Prepare a 25-$cm^2$ or a 75-$cm^2$ culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.

2. 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).

3. 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 operations from this point on should be carried out under strict aseptic conditions.

4. Transfer the vial contents to a centrifuge tube containing 9.0 ml of complete culture medium and centrifuge the cell suspension at approximately 275 x g +/- 125 x g for 5 to 7 minutes.

5. Discard the supernatant and resuspend the cells in fresh growth medium. Count the cells and seed new culture flasks at a density of 5,000 viable cells per $cm^2$.

6. Incubate the culture at 37°C in a suitable incubator.

7. A 5% $CO_2$/95% air atmosphere is recommended if using the medium described on this product sheet.

**Subculturing procedure:**

**Protocol:**

1. Passage immortalized adipose-derived MSCs when the culture has reached approximately 80% confluence.

2. Warm both the Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) and the Trypsin Neutralizing Solution (ATCC PCS-999-004) to room temperature prior to dissociation. Warm the complete growth medium to 37°C prior to use with the cells.

3. For each flask, carefully aspirate the spent media without disturbing the monolayer.

4. Rinse the cell layer one time with 3 to 5 mL D-PBS (ATCC 30-2200) to remove residual medium.

5. Add prewarmed trypsin-EDTA solution (1 to 2 mL for every 25 $cm^2$) to each flask.

6. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer.

7. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 3 to 5 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.

8. When the majority of cells appear to have detached, quickly add an equal volume of the Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.

9. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.

10. Add 3 to 5 mL D-PBS (ATCC 30-2200) to the tissue culture flask to collect any additional cells that might have been left behind.

11. Transfer the cell/D-PBS suspension to the centrifuge tube containing the trypsin-EDTA- dissociated cells.

12. Repeat steps 10 and 11 as needed until all cells have been collected from the flask.

13. Centrifuge the cells at 275 x g +/- 125 x g for 5 minutes.

14. Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, prewarmed, complete growth medium.

15. Count the cells and seed new culture flasks at a density of 5,000 viable cells per $cm^2$.

16. Place newly seeded flasks in a 37°C, 5% CO2 incubator for at least 24 to 48 hours before processing the cells further.

**Cell seeding density:** 5,000 viable cells per $cm^2$
**Medium renewal:** every 2 to 3 days
**Reagents for cryopreservation:** 90% Complete growth media; DMSO, 10%

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: ASC52telo, hTERT immortalized adipose derived Mesenchymal stem cells (ATCC SCRC-4000)

## References

References and other information relating to this material are available at www.atcc.org.

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## Revision

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