L-WRN

CRL-3276™

Description

L-WRN is a fibroblast-like cell that was isolated from the areolar of a male mouse. This cell line was deposited by T Stappenbeck (Washington University) in 2013.
Organism: *Mus musculus*, mouse
Tissue: Subcutaneous connective tissue; Adipose; Areolar
Age: 100 days
Gender: Male
Morphology: fibroblast
Growth properties: Adherent
Volume: 1.0 mL

Storage Conditions

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

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.

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 ATCC-formulated Dulbecco's Modified Eagle's Medium, (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium:

- 0.5 mg/mL G-418
- 0.5 mg/mL hygromycin B
- fetal bovine serum to a final concentration of 10%

Note: Because of limited stability, the G418 should be added to an aliquot of the above complete culture medium fresh prior to performing fluid additions. Complete medium with G418 is stable for 7 days in culture. Use 10 µL G418 per 1 mL culture medium.

Start-up Medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, (ATCC 30-2002). To make the start-up medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

Handling Procedure:

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.

1. Pre-warm growth medium (the Start-up Medium without G418 and without Hygromycin B) in flask at 37°C.
2. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring 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 of the operations from this point on should be carried out under strict aseptic conditions.
4. Transfer the vial contents to the culture pre-warmed flask with medium. Because cells are very fragile after thawing, centrifugation and excess pipetting must be avoided.

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.
6. After 24 hours, change medium with complete growth medium, which contains G418 and Hygromycin B. Centrifuge at approximately 150-400 x g for 8-10 minutes.

Note: Cells may appear to be very low density (≤ 5% confluence) one day after seeding but will typically grow to high density and display more uniform morphology within a week.

Subculture when culture is subconfluent or proceed with protocol to prepare conditioned medium.

Recovery Notes:

- Do NOT centrifuge cells to remove DMSO. Cells are very fragile after thawing.
- Dilute the thawed vial of cells in the start-up medium prepared WITHOUT G418 or Hygromycin B.
- On day 1, perform complete fluid change with complete growth medium containing G418 and Hygromycin B.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium. Briefly rinse the cell layer with Ca^{++}/Mg^{++} free Dulbecco's phosphate-buffered saline (D-PBS) (ATCC 30-2200) or 0.25% Trypsin – 0.53mM EDTA (ATCC 30-2101) solution to remove all traces of serum which contains trypsin inhibitor.
2. 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.

3. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently

pipetting.

4. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C.

Protocol for Wnt-3A, R-spondin and Noggin Conditioned Medium:

1. Split the cells 1:10 in culture medium (without G418 and Hygromycin B to prevent carryover of drugs in the conditioned medium) and seed 25 mL cell suspension into T-150 flasks
2. Incubate the flasks for 3 or 4 days or until the cells become over-confluent and a number of cell aggregates come off.
3. Remove medium and rinse flasks with 10 mL medium and discard rinse.
4. Add 25 mL fresh medium and incubate flasks for 24 hours.
5. Remove the medium to a centrifuge tube. Add new medium to the flasks. Centrifuge the conditioned medium at 2000 x g for 5 minutes and decant supernatant into a 1 L bottle. Store the conditioned medium at 4°C. This is the first batch of medium.
6. Every 24 hours, collect 2^{nd}, 3^{rd} and 4^{th} conditioned medium. Centrifuge and add to same same bottle.
7. After the 4^{th} collection, add an equal volume of fresh medium to the bottle (final concentration: 50%), mix well and aliquot media into 50 mL centrifuge tubes and store at -20°C.
8. Collect 5^{th} to 8^{th} and 9^{th} to 12^{th} conditioned media, if desired.

Recommended Sub Culturing seeding density: A subculturing density of 1.5 x 10^4 to 3.0 x 10^4 viable cells/cm2 is recommended.

Medium Renewal: Every 2 to 3 days.

Reagents for cryopreservation: Complete growth medium supplemented with 20% (v/v) fetal bovine serum (ATCC 30-2020) and 10% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: L-WRN (ATCC CRL-3276)

References

References and other information relating to this material are available at

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