C2C12 Scrambled

CRL-3419™

Description

C2C12 Scrambled is a cell line with myoblast morphology that was isolated in 2013 from the skeletal muscle of a mouse. The cell line was created by transfecting C2C12 cells with scrambled shRNA. The scrambled shRNA is maintained in the cell line by growing the cells with 2 µg/ml puromycin. Cells show same levels of dysferlin expression as wild type cells. This is the control cell line for C2C12 Clone 5 DYS shRNA (ATCC CRL-3418). This cell line can be used to study membrane repair and muscle regeneration mechanisms.

Organism: *Mus musculus*, mouse
Tissue: Skeletal muscle
Age: adult
Morphology: myoblast
Growth properties: Adherent
Cells per vial: Approximately 2.0 x 10^6
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 1

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.

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 Dulbecco's Modified Eagle's Medium (DMEM; ATCC 30-2002). To make the complete growth medium for the first 3 to 5 days of culture, add the following components to the base medium: fetal bovine serum (FBS; ATCC 30-2020) to a final concentration of 10% and 0.5 µg/ml puromycin. After 3-5 days, use DMEM + 10% FBS with 2 µg/ml puromycin in order to maintain the presence of the DYS shRNA.

Handling Procedure:

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 phase 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 150-400 x *g* for 8 to 12 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% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 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 DPBS (ATCC catalog # 30-2200) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL 0.25% (w/v) Trypsin- 0.53 mM 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.
   Cultures can be established between 2.5 x 10^4 and 4.0 x 10^4 viable cells/cm^2.
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 2.0 x 10^4 and 3.8 x 10^5 cell/cm^2.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week

Reagents for cryopreservation: Complete growth medium supplemented with 5% (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: C2C12 Scrambled (ATCC CRL-3419)

References

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

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