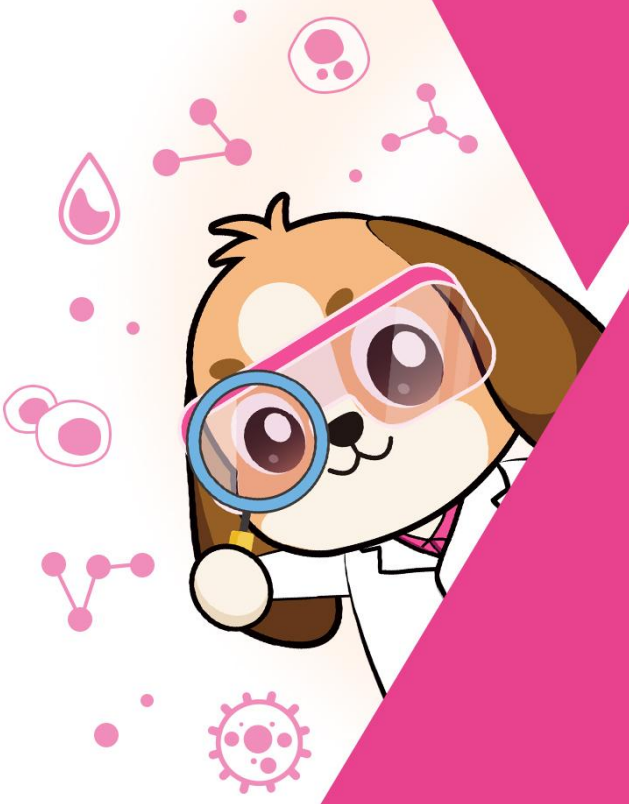
cyagen | OriCell™

User Manual

OriCell™ SD Rat Tendon Stem Cells

Catalog No. RASTA-01001



We help you discover life

Introduction

Rat tendon stem cells are a type of mesenchymal stem cell derived from rat tendon tissue, characterized by abilities such as self-renewal, multi-lineage differentiation, and colony formation. They are typically isolated from tendon tissue using a mixed enzymatic digestion method involving collagenase and neutral protease, and then cultured and expanded in a specialized tendon stem cell culture medium.

Tendon stem cells have the potential to differentiate into multiple lineages, including tendon cells (tenocytes), chondrocytes, osteocytes, and adipocytes. They play a crucial role in tendon injury repair by promoting the regeneration of tendon tissue and the restoration of its function. The normal self-renewal and differentiation of tendon stem cells are essential for maintaining the structure and function of tendons. Abnormal differentiation of these cells may lead to tendon disorders, such as chondrification, ossification, and adipogenesis within tendon tissue, which can adversely affect tendon healing.

Rat tendon stem cells, as a unique stem cell population, have become an important cellular resource in tendon tissue engineering and related disease treatment research due to their distinctive differentiation potential and key role in tendon repair.

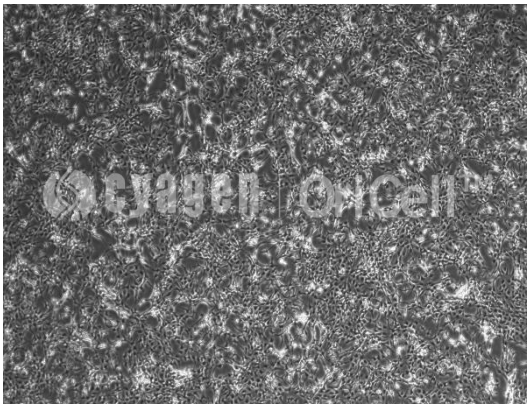
Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

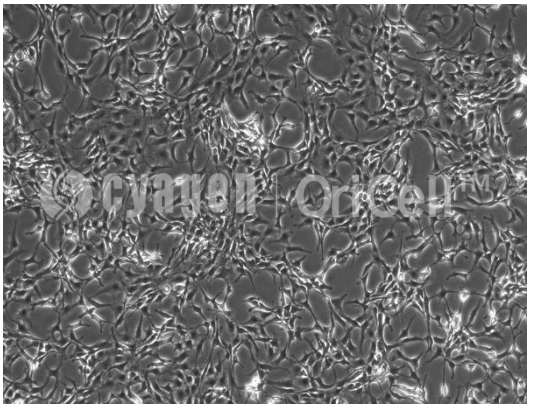
When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Name	OriCell™ SD Rat Tendon Stem Cells
Catalog Number	RASTA-01001
Amount of Cells	1×10^6 cells/vial
Passage Number	P2
Storage at	Liquid Nitrogen (-196°C)

The Shape of OriCell™ SD Rat Tendon Stem Cells





QC

- Pass the detection of bacteria, fungi, mycoplasma and endotoxins.
- Pass the viability examination. The viable rates is higher than 80%.
- The cell doubling time is less than 72 hours.
- Flow cytometry showed that CD29, CD44 and CD90 are positive (>70%), while CD34, CD45 and CD11b are negative (<5%).
- The cells can be induced to differentiate into osteoblasts, adipocytes, chondrocytes, etc.

Please refer to "COA" for details.

General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Please follow the instructions.
3. Use suitable and reliable consumables and reagents.
4. Rat tendon stem cells have limited ability to proliferate in vitro and cannot maintain their differentiation potential for a long time. OriCell™ SD Rat Tendon Stem Cells can be passaged for more than 5 times and still maintain all indicators qualified. But we always recommend using lower generation cells for scientific research.
5. Usually the inoculation density of rat tendon stem cells is (2.5~4) $\times 10^4$ live cells/cm^2.

Note: **The cryopreservation solution of this product contains DMSO, which has potential risks. Please handle it carefully.**

Thawing and Establishing of Cells

Materials Required

- OriCell™ SD Rat Tendon Stem Cells (Cat. No.: RASTA-01001)
- OriCell™ Complete Medium For Rat Tendon Stem Cells (Cat. No.: RAXTA-90011)

Steps

Note: **If the cells are thawed within 24 hours of receipt, they can be stored at -80°C. If storing for more than 24 hours, please keep them in liquid nitrogen. Before thawing, remove the cells from liquid nitrogen about 10 minutes in advance and place them at -80°C to allow any residual liquid nitrogen in the tube to evaporate.**

1. Preheat the water bath at 37°C.
2. Warm the complete medium to 37°C.
3. Add at least 5 mL of complete medium to a 15 mL centrifuge tube for use.
4. Remove the cells from the -80°C freezer, immerse the cryotube in the 37°C water bath, and gently agitate to thaw the cryopreservation solution evenly.

   Note: **During the thawing process, the cryotube must be shaken to ensure that the solution thaws quickly and evenly.**

5. When shaking, keep the cryotube upright and avoid immersing the tube cap in water to prevent contamination.
6. When ice crystals approximately 2 mm in diameter remain, stop the water bath and continue gently agitating the tube until the ice is completely melted.
7. Wipe the outer surface of the cryotube with 75% ethanol.
8. Inside an ultraclean bench, open the cryopreservation tube and transfer the cell suspension into the prepared centrifuge tube using a Pasteur pipette.
9. Rinse the cryotube once with 1 mL of complete medium to collect residual cells and add to the centrifuge tube.
10. Centrifuge the cell suspension at 250×g for 4 minutes.
11. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium and gently pipette up and down to resuspend the cell pellet thoroughly.
12. Seed the cells into a T25 flask or an equivalent culture vessel. Add sufficient complete medium to ensure a total volume of no less than 5 mL in the T25 flask.
13. Gently resuspend the cells and incubate in a humidified 37°C incubator with 5% CO_2.

    Note: **Avoid moving or observing the cells within 2 hours after seeding, as this may impair adhesion and cause poor morphology, clumping, or uneven attachment.**

14. The next day, observe the cell status and either replace the medium with fresh complete medium or passage the cells as needed.

Note: **If excessive floating cells or other abnormalities are observed, investigate promptly and contact us for support.**

15. Change the complete medium every 2 days until the cells reach approximately 90% confluence, at which point passaging should be performed.

Passaging of Cells

Materials Required

- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No.: TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Complete Medium For Rat Tendon Stem Cells (Cat. No.: RAXTA-90011)

Steps

1. Prewarm the complete medium and trypsin to 37°C.
2. Discard the culture medium from the culture vessel.
3. Wash the cells twice with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask). Wash gently but thoroughly, then remove the PBS.
4. Add trypsin solution (approximately 1.5 mL for T25 flask and 3 mL for T75 flask), and quickly spread to ensure full contact with the cells.
5. Observe the cells under a microscope. When approximately 70–80% of cells are rounded and detached, gently tap the outer wall of the culture vessel to dislodge the cells.
6. Immediately add complete medium (approximately 3 mL for T25 flask and 6 mL for T75 flask), then gently shake the vessel to mix the medium and trypsin and stop digestion.
7. Aspirate the cell suspension by pipetting along the bottom surface of the vessel several times to ensure maximum cell recovery.

Note: **Pipetting should be gentle to avoid cell damage.**

8. Transfer the cell suspension to a centrifuge tube. Rinse the culture vessel once with PBS (approximately 3 mL for T25 flask and 6 mL for T75 flask) to collect residual cells, and add to the centrifuge tube.
9. Centrifuge the cell suspension at 250 × g for 4 minutes.
10. Discard the supernatant after centrifugation. Add 2 mL of complete medium and gently pipette up and down to resuspend the cell pellet thoroughly.
11. Seed the cells into an appropriate culture vessel at a density of (2.5–4) × 10^4 live cells/cm^2, or adjust the seeding density according to actual cell growth.

    Note: **OriCell™ SD Rat Tendon Stem Cells typically have a passage ratio of 1:3 and reach confluence within 72 hours.**

12. Gently resuspend the cells and incubate in a humidified 37°C incubator with 5% CO_2.
13. Change the complete medium every 2 days until the cells reach approximately 90% confluence, at which point passaging or cryopreservation should be performed.

    Note: **Under normal conditions, OriCell™ SD Rat Tendon Stem Cells grow within 72 hours per passage, and there is no need to change the medium. Frequent medium changes may disrupt the cellular microenvironment.**

Cryopreservation of Cells

Materials Required

- OriCell™ NCR Protein-Free Cryopreservation Medium For General Use (Cat. No.: NCPF-10001)
- OriCell™ Cryopreservation Medium For General Use (Cat. No.: CYRO-10001)

Steps

1. If you choose OriCell™ Cryopreservation Medium For General Use, please put the freezing containers in the refrigerator at 4°C before next process.
2. Cryopreserve cells when they reach an appropriate density suitable for passaging.
3. For the cell digestion procedure, refer to steps 1-9 under the section titled “Passaging of Cells”.
4. Resuspend the cells uniformly in an appropriate volume of cryopreservation medium, then centrifuge and remove the supernatant.
5. Aliquot the cells into cryopreservation tubes according to the desired volume or cell number.
6. If using OriCell™ Cryopreservation Medium for General Use, place the cryotubes into freezing containers, then transfer the containers to a -80°C freezer. If using OriCell™ NCR Protein-Free Cryopreservation Medium for General Use, place the cryotubes directly into the -80°C freezer.

   Note: **Do not open the freezer door during the first 4 hours of freezing, as this will significantly reduce cell viability.**

7. After 8 hours, transfer the cells to liquid nitrogen for long-term storage.

   Note: **Storage at -80°C should not exceed 48 hours.**