Rev. 0

Rat Splenic Fibroblasts (RSF)

Catalog #R5530

Cell Specification

Orchestrated by a specialized network of resident splenic fibroblasts (SF), the spleen coordinates systemic immune surveillance within its white pulp and maintains blood homeostasis via red pulp-mediated filtration. Through these distinct stromal and compartmentalized functions, it effectively clears blood-borne pathogens while initiating robust adaptive immune responses. Splenic fibroblasts comprise a heterogeneous group of mesenchymal stromal cells critical for organ compartmentalization, structural maintenance, and immune regulation. Splenic fibroblasts drive a diverse array of functions, represented by specialized subsets such as fibroblastic reticular cells (FRCs), red pulp fibroblasts, and marginal reticular cells [1, 2]. Fibroblastic reticular cells (FRCs) drive stromal network organization, actively providing the cues necessary to orchestrate successful T cell activation [3]. Red pulp fibroblasts maintain the spleen's structural integrity, support blood filtration and immune cell reserves, and provide a microenvironment for red blood cell turnover. Positioned at the marginal zone, marginal reticular cells (MRCs) serve as structural organizers and gatekeepers between the spleen's red and white pulp. Rat splenic fibroblasts (RSF) can be used to study immune cell interactions with the spleen's microenvironment and the differentiation and plasticity of stromal cells.

RSF from ScienCell Research Laboratories are isolated from rat spleen. RSF are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. RSF are characterized by their spindle-shaped morphology and immunofluorescence with antibody specific to fibronectin. RSF are negative for mycoplasma, bacteria, yeast, and fungi. RSF are guaranteed to further expand for 5 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Fibroblast Medium-2 (FM-2, Cat. #2331) for culturing RSF *in vitro*.

Product Use

RSF are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Pezoldt J, Wiechers C, Erhard F, Rand U, Bulat T, Beckstette M, Brendolan A, Huehn J, Kalinke U, Mueller M, Strobl B, Deplancke B, Čičin-Šain L, Sitnik K. (2021). Single-cell transcriptional profiling of splenic fibroblasts reveals subset-specific innate immune signatures in homeostasis and during viral infection. *Communications Biology*, *4*(1), 1355.
[2] den Haan J, Mebius R, Kraal G. (2012). Stromal cells of the mouse spleen. *Frontiers in Immunology*, *3*, 201.
[3] Sng XYX, Voigt V, Schuster IS *et al.* (2026) Fibroblastic reticular cells direct the initiation of T cell responses via CD44. *Nature* 651, 752–762.

Instructions for culturing primary cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-$_L$-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). To obtain a 2 µg/cm^2 poly-$_L$-lysine-coated culture vessel, add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-$_L$-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add 20 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-$_L$-lysine-coated culture vessel.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-$_L$-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. Do not disturb the culture for at least 16 hours after initiation. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
2. Prepare poly-$_L$-lysine-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, 0.05% (T/E, Cat. #0183), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 5 ml DPBS and 5 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

   Note: We recommend using ScienCell 0.05% T/E solution which is optimized to minimize cell damage due to over trypsinization. If 0.25% T/E solution (Cat. #0103) is used, then 9 ml of DPBS and 1 ml of 0.25% T/E solution should be used.

   Caution: Do NOT use undiluted trypsin when subculturing primary cells.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Once cells completely round up, transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Gently resuspend cells in culture medium.
12. Count and plate cells in a new poly-$_L$-lysine-coated culture vessel with the recommended cell density. A seeding density of 5,000 cells/cm^2 is recommended.

    Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling animal-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.